PLANT PHYSIOLOGY

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IVa

HOMER LEROY SHANTZ

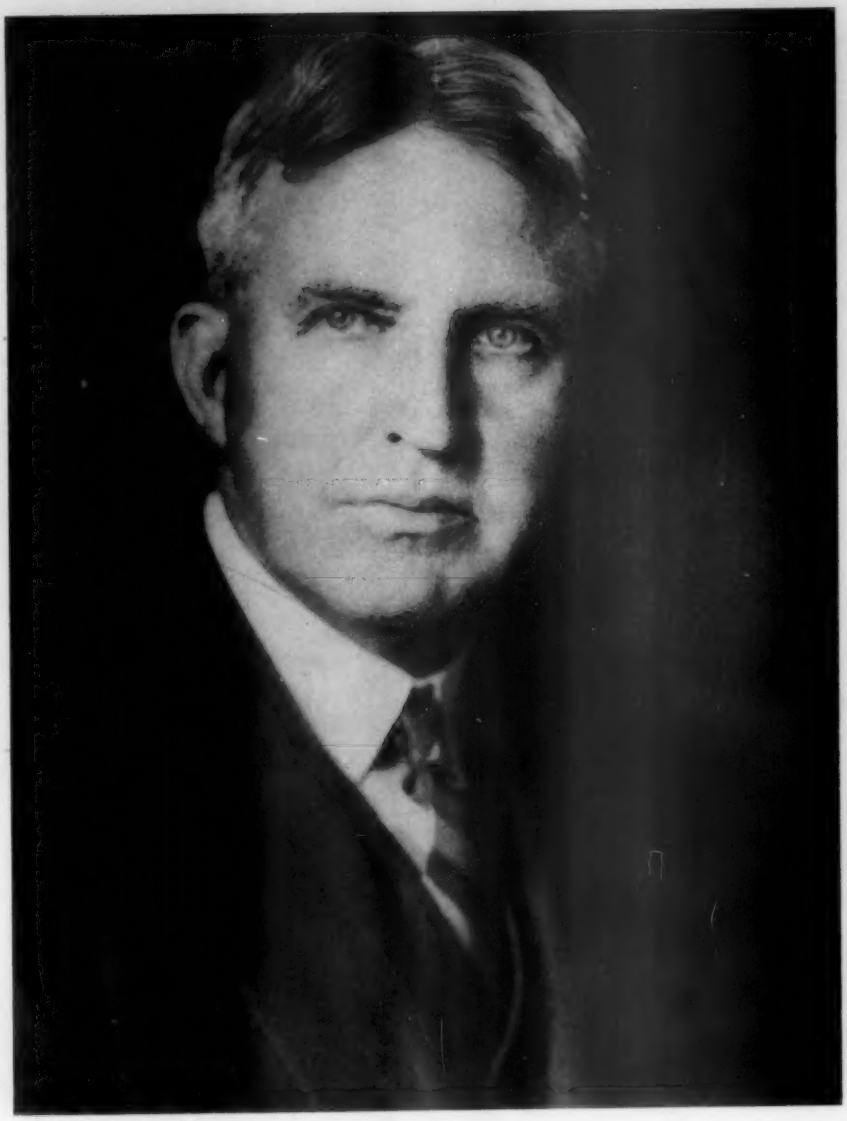
IN CELEBRATION OF

HIS SEVENTIETH BIRTHDAY

JANUARY 24, 1946

ERRATA

Vol. 21, no. 1, cover, second line under contents, ''Micorrhizae'' read Mycorrhizae.
Page 4, line 30, ''mitochrondria'' read mitochondria.
Page 35, line 23, ''to'' read when.
Page 87, figure 5, legend, ''inorganic-nitrogen'' read protein-nitrogen.
Page 212, line 32, ''*Adropogon*'' read *Andropogon*.
Vol. 20, page 163, no. 17, ''Findlay, A.'' read ————; no. 18, ''————,'' read Findlay, A.
Vol. 20, page 690, line 4 of table I, ''unpuried'' read unpurified.
Vol. 20, page 689, line 9, ''leaves'' read leaves.
Vol. 21, page 372, lines 1–2, ''In 1915 a study . . . was published by MacDougal, Long, and Brown.'' It was Dr. Edmond Ray Long who collaborated in the study. Dr. Frances Long became associated with the Desert Laboratory in 1917. The results of her efficient collaboration in the study of long-lived cells in the medullary tracts of cacti were published in 1927.



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RODNEY BEECHER HARVEY
MAY 26, 1890—NOVEMBER 4, 1945

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IN MEMORIAM

RODNEY BEECHER HARVEY

MAY 26, 1890—NOV. 4, 1945

Sunday morning, November 4th, 1945, marked the passing of one of the outstanding Plant Physiologists of this generation. DR. RODNEY B. HARVEY, professor of Plant Physiology, Agricultural Botany, and Horticulture, died of a heart attack in the University of Minnesota Hospital after a very brief illness.

DR. RODNEY BEECHER HARVEY was born at Monroeville, Allen County, Indiana, May 26, 1890. He entered Purdue University in 1910, and was graduated in 1912. On June 17, 1916 DR. HARVEY married HELEN M. WHITTIER who survives him. Their five children are: HALE M. WHITTIER (deceased), RODNEY BRYCE, RHODA BEATRICE, HELEN ELIZABETH, AND ELEANOR W. HARVEY.

In 1912 DR. HARVEY was appointed Assistant Botanist at the large pharmaceutical manufacturing laboratory of the Eli Lilly Company of Indianapolis. In 1913 he entered the University of Michigan where the degree of Bachelor of Science was granted in February, 1915; he continued as a graduate student in Plant Physiology for the following semester. In June, 1915 he entered the University of Chicago as a candidate for the degree of Doctor of Philosophy.

In August of 1915, DR. HARVEY entered the U. S. Bureau of Chemistry as pharmacognocist, but was transferred within a month to work in Plant Physiology under DR. RODNEY H. TRUE in the Office of Plant Physiological and Fermentation Investigations in the Bureau of Plant Industry. His former professor, DR. WILLIAM CROCKER, came to this laboratory to do research for a year. During the period from 1915 to 1920 the influence of DRS. TRUE and CROCKER led HARVEY into research on the mineral nutrition of plants, salt absorption, permeability studies, enzyme activity, respiration, and winter hardiness. The broad field of research in progress in this laboratory thoroughly acquainted him with research methods and led him to publish on all of these special topics. DR. HARVEY also had the valuable friendship at this period of his career of DR. ERWIN F. SMITH and through this connection he undertook some researches in vegetable pathology, chiefly of a physiological nature. Under the influence of DR. TRUE he started a bibliographic index of his subject, which had been continued until his death and now represents the most complete bibliography of its kind. Part of this compilation has been published in book form.

In 1917 DR. HARVEY returned to the University of Chicago as a Fellow, and continued research for the Ph. D. degree which was granted in 1918. His thesis was on the winter hardiness of plants. As a result of this work,

he introduced the Artificial Winter Test for determining the varietal hardiness of plants, a method which has been much used in northern countries by plant breeders to produce hardy plant varieties. Interest in this development was largely responsible for his invitation to join the staff of the University of Minnesota in 1920 where he became an outstanding research worker and authority upon this subject. He and the many students who studied with him published a great number of research findings on winter hardiness. Since 1931 he had been Professor of Plant Physiology, Agricultural Botany, and Horticulture.

DR. HARVEY'S interest in research on fruits and vegetables came through his knowledge of the low temperature relations of plants and cold storage problems. Into the solution of these problems he threw all of his energy and experience and since 1924, many improvements in fruit processing have come from his laboratory. Ethylene ripening was made a subject of intensive research, leading to the demonstration that ethylene is produced naturally in fruits and vegetables during a process of ripening and blanching. The obnoxious flavors from spoiled fruit were found to be transferred to healthy fruits stored with them, and the use of cellulose wrappings and sterilization were found to decrease spoilage. The flavors of fruits depending upon minute quantities of esters and other such volatile compounds were found to be capable of manipulation, so that new flavors could be produced in stored fruit, or the flavors enhanced artificially. Considerable research was completed on the physiology of plant pigments and their transformations.

DR. HARVEY'S range of interests extended over the whole field of plant physiological research. He developed research on the light intensities required by plants, and was the first one to grow plants from generation to generation entirely in artificial light. DR. HARVEY'S demonstration of light requirements for the blooming of plants has led to widespread use of electric illumination in the study of plant reactions to light including the use of artificial light to cause the blooming of flowers out of their usual season, as well as in forcing seedlings for plant breeding purposes. In studies of seed germination DR. HARVEY made a most complete survey of the subjects of the length of the rest period of cereal seeds in relation to their storage conditions, methods of breaking dormancy in forest tree seeds, and the testing of seed viability.

In the practical applications of Plant Physiology in agriculture, DR. HARVEY developed a series of new chemicals for use in the control of weeds. He introduced the use of the cyanates, particularly sodium and ammonium sulfocyanates, which are of value as fertilizers after their toxic action has been destroyed by bacterial decomposition in the soil, yielding nitrogenous fertilizers. Recently the xanthates were found to be of use as herbcides by his laboratory. He also found that persulfates and perchlorates had value as herbicides under certain conditions. At the time of his sudden death, DR. HARVEY was carrying on investigations of the effectiveness of certain hormones in weed eradication.

DR. HARVEY was one of the founders of the American Society of Plant Physiologists in 1924 and he served as its first secretary. In 1931 he became vice-president of the society, and was elected president in 1936. DR. HARVEY also was largely responsible for the formation of the Minnesota Academy of Science, an active organization which now numbers some five to six hundred members. In 1927–28 he was a fellow of the Guggenheim Foundation, studying at Cambridge University in the School of Botany with DR. F. F. BLACKMAN, and at the Low Temperature Research Station with SIR WILLIAM HARDY, PROFESSOR FRANKLIN KIDD, and PROFESSOR CYRIL WEST. He also studied at Bonn University with DR. ERNEST SCHAFFNIT, and at Leningrad with DR. NICOLA A. MAXIMOW. He was further commissioned by the Guggenheim Foundation to make a survey of research in progress in the Agricultural Experiment Stations throughout Russia.

For the year 1936–37 DR. HARVEY was director of the Florida Citrus Research Laboratory at Dunedin, Florida. This laboratory was founded to conduct research on fruit processing in connection with industrial and state institutions. In his industrial connections DR. HARVEY has been associated with The Food Machinery Company, Ind., the B. C. Skinner Company, Pacific Coast Distributors, and the United Fresh Fruit and Vegetable Association in connection with fruit processing; with the Carbide and Carbon Chemicals Company and the United Fruit Company in fruit ripening; with The American Potash Institute, the Kippers Coke Company, and the American Cyanimide Company in the development of herbicides; and with many other industrial concerns whose facilities have made possible the practical commercial development of the processes which he has developed. In 1942–43 he was director of the Division of Industrial Microbiology of General Mills of Minneapolis.

DR. HARVEY was the author of three books in the field of Plant Physiology, and of nearly two hundred bulletins and scientific papers. For many years he had the hobby of collecting historical materials relating to Plant Physiology and the History of Botany. He also had collected about 500 portraits of research men, laboratories, and student groups of historical interest. Included among the portraits were 67 paintings by the well-known Russian artist, STREBLOV, of life size portrait busts of plant physiologists.

At the time of his death, DR. HARVEY had just completed 25 years of service in plant physiology and related subjects at the University of Minnesota. In addition to his work as a teacher at the University and research worker in the Agricultural Experiment Station, he had perfected a large number of new methods for processing fresh fruits and vegetables which have been of great value to the industry and to the consuming public. Several of his processes had been patented, among which were the use of ethylene gas for hastening fruit ripening; enhancing the color of citrus fruits; x-ray methods of inspecting fruits and vegetables for internal defects and diseases; control of the flavor of fruits; fruit preservation and steriliza-

tion; and the blanching of citrus fruits to remove the green color from varieties that are of a greenish color when ripe. DR. HARVEY'S process of ripening fruits by ethylene gas has had world wide use, having been applied to fruits such as bananas, tomatoes, honey dew melons, and tropical fruits which must be shipped in the green condition and ripened at destination. Practically all melons shipped from California to eastern ports are ripened with ethylene. The greater portion of the tomatoes shipped from California, Florida, and Mexico to the north in winter are ethylene treated. This process in combination with organic sterilizing agents and the use of cellophane wrappings and waxing processes has greatly improved the quality of fresh fruits and decreased spoilage from diseases incident to transport over long distances. DR. HARVEY had only recently perfected a process for waxing and polishing fruits which obviates the use of brushing and thus avoids injuries to the peel of soft fruits such as tomatoes, tangerines, pears, and apples. Bananas can be waxed and polished while in the bunch, to allow them to be transported over greater distances without shrinkage and spoilage. These processes have been a boon to the fruit and vegetable trade as well as to the consumer, and they have been used in many countries outside of the United States.

DR. HARVEY'S work has been recognized in this country by the award of the honorary degree of Doctor of Science by Purdue University in June, 1939. He became a starred scientist in American Men of Science in 1933. He was also a corresponding member of the Botanical Society of Czechoslovakia and of the graduate faculty of the University of Madras, India. He was a fellow of the American Association for the Advancement of Science and a member of the following societies: The American Chemical Society, The Botanical Society of America, the American Pathological Society, American Society of Agronomy, American Society for Horticultural Science, American Society of Plant Physiologists, Minnesota Academy of Science, and the American Genetics Association. He belonged to the following fraternities: Sigma Xi, Gamma Alpha, Alpha Zeta, Phi Lambda Upsilon, and Gamma Sigma Delta.

Vol. 21 No. 1

PLANT PHYSIOLOGY

JANUARY, 1946

CRITERIA OF NUTRITIVE RELATIONS OF FUNGI AND SEED-PLANTS IN MYCORRHIZAE

D. T. MacDougal and Jean Dufrenoy

Nutritive engagements of plants

The senior author has recently had occasion to scan nearly eight hundred abstracts of the contributions which constitute the main part of the literature of symbiosis in plants. This material was made available by the kindness of Dr. A. P. Kelley, the prospective author of a volume of Mycorrhizae in the New Series of Plant Science being produced by Chronica Botanica.

A very high proportion of the contributors base conclusions as to nutritive relations upon morphological characters, the results of germination tests, or aspects of life histories. It is becoming increasingly evident that plants anatomically engaged present examples ranging from destructive or pathogenic parasitism through intermediate stages of unbalance to stabilized symbiosis, further to associations incidentally or slightly beneficial to one or both elements; the advantages or disadvantages of the adherence of the two species are in some cases no more than mechanical support. In addition plants set free substances in the soil or substratum which may be toxic or beneficial to other species in proximity. It is obvious therefore that the category of terms currently applied to associations (facultative–obligate parasitism, commensalism, etc.) are without exact meaning, although of some convenience in text-books and general discussions.

The determining features of nutritive relations are those of the origination, flow, and ultimate disposal of material; the minor feature is that of the resultant alterations in tissue structure, cytological departures, and modifications of metabolism. It is therefore plainly evident that the nature of the association of two species may be determined with finality only on cytological and cytochemical evidence. Information included in the last category will be of value only if the main features of the chain of metabolic processes are adequately delineated. Thus, for example, in many cases of parasitism the invading organs of the parasite, generally with its vacuoles with a higher osmotic potential than those of the host, will cause the translocation of phosphorylated sugars to the cells of the host nearest the para-

site; some of this material will be drawn into the parasite, but a surplus may accumulate and be synthesized into starch. Concentration of material may result in renewed growth of the tissues of the host; this, with the surplus starch, may make it deceptively apparent that the presence of the fungus is beneficial to the host.

The authors have recently made a study of a terrestrial orchid with green leaves, a chlorophylless orchid with no roots, and a pine tree, all with highly developed mycorrhizae features (**6**). The chain of metabolic processes and the compounds translocated from the fungus to the higher plants were found to be identical.

The soil fungi have an independent, non-measurable existence in the soil, and a minute fraction of their mycelia are engaged in the mycorrhizae. The material received by the higher plants is of great importance to their development and continued existence; full maturity may not be reached without such a supply, a condition applicable to probably more than nine-tenths of all seed plants. By the application of pigeon-holing, or of empirical terms mentioned above, this enormous number of species might be said to be parasitic on soil-fungi, which, however, would be simply a specious statement of the general inter-dependence of plants under natural conditions.

While the general features of symbiosis between fungi and seed plants as exemplified by mycorrhizae have been the subject of many contributions, no well-established conclusions have been reached as to the beneficial results of the association to the fungus partner. On the other hand the processes by which electrolytes and organic material are absorbed from the soil by mycelia, elaborated into compounds fundamental to protoplasm, and translocated to the higher plant are recognizable as described in later paragraphs.

Extended observations and contributions by other workers subsequent to our earlier paper make it possible to clarify some of the tentative conclusions advanced as to the synthesis and translocation of the auxins, carbohydrates, nucleo-proteins, phospholipids, etc., which constitute such important features of the chain of metabolic processes. The more nearly complete outline of the symbiotic relations presented appears to call for and to justify some speculation as to their origination.

In the engagement of fungi with the underground organs, roots, or stems, hyphae may form heavy felts on the surface, sending sparse branches into epidermal or cortical cells; or these branches may traverse the middle lamella so that a network encloses the protoplast. In any case, hyphal branches penetrate to the vacuoles amidst which they develop dense clumps or, in other types, large blasts at the ends of the hyphae. The stage at which translocation from the fungus to the cell of the higher plant occurs as well as the incidental cytological features vary widely.

The survival or behavior of the occupied cells—symbiosis or parasitism—is determined by the balance between the agencies which activate hydrogen from such metabolites as sugar and the agencies which activate atmospheric

oxygen so that the latter will accept the former. The activation of hydrogen or its transfer from the level occupied in sugar, for example, to a level at which it may be accepted by activated oxygen, depends upon dehydrogenases which comprise compounds of C, N, P, Fe, and S of high molecular weight and specialized structure, presenting active groups such as sulfhydril. [See (**9**) for discussion of increased absorption of salts from soil, respiration, and fermentation in pine root segments.]

This dispersion of dehydrogenases entails the decompensation of aerobic respiration as evidenced by the progressive dehydrogenation of the phenolic compounds in solution in the vacuoles into quinone polymers.

So long as the dehydrogenases perform normally in aerobic respiration they re-hydrogenate phenolic compounds back to their original state as soon as they have released hydrogen to oxygen, thereby being dehydrogenated to quinoids. The continued ramification of hyphae may proceed in such a field. The dispersion of the dehydrogenases (complex compounds of C, N, P, and S) is, as noted, followed by the more complete oxidation and polymerization of the quinoids into gummy tannin masses with fixation of the proteins present. These visible masses constitute a barrier limiting the extension of the hyphal branches.

Cytochemical methods of analysis of nutritive relations of mycorrhizal elements

Chief interest centers in the arrangements of the structural constituents of the active cells of the cortex and especially the localization of the phosphorus complexes of the dehydrogenase system and the copper-protein systems acting as polyphenol oxidase in the derivation of the quinoids.

Valuable information may be obtained by vital staining to localize important cell components and by methods of fixation which give immediate reactions parallel to the changes which follow slowly a decompensated respiration in living cells.

Vital staining

In the employment of vital stains it is important to note that the color reactions in the vacuolar solutions are of determinative value only so long as the vacuole is limited or enclosed by living cytoplasm.

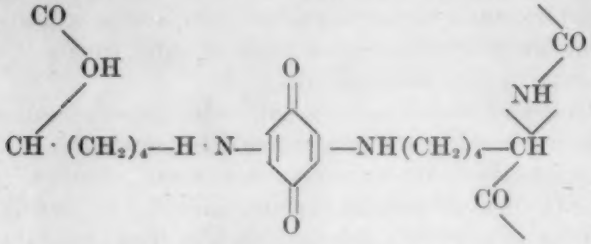
When freehand sections of living pine mycorrhizae are immersed in a solution of neutral red, isotonic and isoionic to the vacuolar solutions, the polyphenols of the vacuoles of both the hyphae and cortex take on a bright red or purplish color.

These polyphenols, following a decompensated respiration, oxidize to quinoids, developing reactive groups which unite with the proteins of the cytoplasm with a resultant fixation or killing, in a manner duplicating death from the action of formaldehyde (**10**).

Formaldehyde and quinone include an active carboxyl group. In acid solutions the aldehyde group of formaldehyde forms a methylol (hydroxymethyl) group with the amino groups of the protein.

$$NH + CH_2O \longrightarrow N—CH_2OH$$

In solutions between pH 5.5 and 7 the quinone penetrates proteins with slow oxidation, polymerization, and fixation of the polymer.



Reliable pictures of the spatial arrangement of cell constituents require, as a prerequisite to staining, fixation by a mixture of formaldehyde and potassium bichromate. As noted above, the formaldehyde supplies the carbonyl group which binds the proteins while the potassium bichromate brings out the "chromatin" effect in which the polyphenols are oxidized by the chromium salts to stable yellow and orange quinoid polymers.

After fixation as above the nucleoproteins in the prochromosomes, the resting nucleus, dividing nucleus, the chromosomes, mitochondria, and other plasts stain a deep black in iron haematoxylin while the cytoplasm remains a faint grey, and the phenolic compounds retain the yellow tinge of the previous chromatin reaction. Alternative to the above reactions, the nucleoproteins in the organs mentioned stain a deep red in acid fuchsin. A further differential reaction may be obtained by the addition of methyl green, which forms a complex with the acid fuchsin, the combination producing shades of purple and blue with the phenolic compounds.

Cells which have become filled with brownish masses of protein-polymerized complexes may be taken to be at the end of their activities in the nutritive system; although the nucleus may still retain an approximately normal structure and appearance as it does in the endodermis and pericycle.

The site of the high phenolase activity which finally results in the above conditions may be found by the use of aromatic amines such as phenylenediamine chlorhydrate, which in the presence of polyphenol oxidase yields a reddish quinoid which fixes the proteins in sections of living material.

Unmasking phosphorus linkages

The great importance of phosphorus in the metabolism of mycorrhizae is suggested by the fact that this element is to be found in compounds, as in ionic PO_4 in vacuolar solutions, and in firmly bound complexes in lipo-nucleo-proteins of the types characterizing mitochrondria and other plasts. A variety of effects may be seen under the microscope after treatment with the well-known molybdenic reagent (**6**).

Water-soluble phosphorus compounds appear immediately as a blue phosphomolybdenic complex; after several hours the more firmly bound

phosphorus in the lipo-nucleo-proteins is unmasked. Mitochondria may be distinguished from other plasts by the further use of acid fuchsin, the specialized combinations in mitochondria becoming bright red while other plasts show a purple tinge. It is notable that while this particularized composition of mitochondria has been demonstrated, yet no well-supported conclusions as to the functions of these organs in the cell have been reached as noted below.

Phosphorus compounds are released in the dispersion of the dehydrogenases in tissues invaded by a parasite and flow toward the parasite. But in mycorrhizal roots of chlorophylless plants, and in cultures of isolated segments of pine roots, all nutritive elements, not only P but also C, N, and S pass from the fungus associate to the root. Since the histological and cytological features of such roots do not differ in any discernible feature from those of the attached mycorrhizae of pines, it may be assumed that the nutritive relations are identical.

A cytochemical appraisal of the cortical cells of underground organs entering into symbiotic relationship with soil fungi, as above described, reveals the fact that C, H, N, O, P, and S, whether absorbed from the soil as electrolytes or as complex organic compounds, are metabolized into the higher groups, carbohydrates, phospholipids, proteins, etc., in the hyphae of the cooperating fungus, and are then translocated to the cortex of the higher plant.

In an earlier publication it was stated that the only carbohydrate recognizable in fungi was glycogen; since this substance was not diffusible it could not be regarded as the immediate source of glucose and starch which appears so abundantly in mycorrhizae. Phosphorylated glycogen, however, can be translocated as is glucose, and this compound may be regarded as the source of the carbohydrates in mycorrhizae, appearing as glucose and starch in the cortex.

Plastids

The plastids show a special relation to certain features of phosphorus metabolism. The amyloplasts present in the cortex, but not in the fungus, synthesize starch from the phosphorylated carbohydrates received from the fungus in mycorrhizae or translocated from neighboring elements. Nucleoproteids are built up in proteoplasts in both hyphae and cortex. Mitochondria, which are characterized by a rod-like form and a sharper molybdenic reaction than other plasts, occur in both hyphae and cortex. As noted above, the part played by these organs of the cell is obscure. It is possible that the reduction of their phosphorus content might coincide with their conversion into amyloplasts, a transformation still in controversy. That the number of these bodies in a cell decreases with maturity, and differentiation coincidental with the other plasts becoming more numerous, can be confirmed. The lateration in position of mitochondria in cells may yet furnish some clue to their cytochemical activity. These bodies are disposed

in strands or crowded around the small vacuoles in meristematic cells. In later stages, when the main vacuole occupies most of the volume of the enlarged cell, mitochondria are disposed at random in the thin peripheral layer of cytoplasm.

Diversity of morphological adjustments

While the chain of processes in which electrolytes and organic compounds are absorbed from the soil, elaborated into proteins, carbohydrates, and phospho-lipidic compounds, which are translocated to the tissues of the higher plant, conform to a general pattern common to all mycorrhizae, the anatomical engagement of the two symbionts shows the widest diversity.

The great majority of these cooperative associations are as the term implies, "fungus-roots"; in a large number of cases, however, the fungus enters the tissues of underground stems, as in many orchids, with modifications of structure carried to the extreme with loss of roots as in Corallorhiza.

The branches of the mycelium which penetrate the underground organs are barred from the stele by the formation of masses of tannin in the endodermis, and the hyphae extend apically in mature or nearly mature cells behind the meristematic region of tips of the roots or stems. In types of mycorrhizae in which the mycelium forms a felted mass around the roots or underground organs the enclosure may be complete and lasting as in Monotropa.

The main roots of pines, the stele of which is diarch, have a capacity for a high rate of elongation so that whitish terminals many centimeters in length are to be seen in the growing season. In a second stage, exfoliation of the cortex ensues, and a cambium layer originates in the pericycle with a subsequent growth in thickness much after the manner of stems. A characteristic morphogenic feature is the origination of myriads of lateral branches which attain a length of only a few millimeters from cell masses of the endodermis and pericycle and are monarch. These rootlets may originate in sterile soil and water cultures, and hence their origination may not be ascribed to the inciting action of the fungus with which they form mycorrhizae; a felted sheath of hyphae so nearly complete that only a very small area of the whitish tip of the rootlet remains visible. The hyphal branches which penetrate the cortex extend internally to the endodermal barrier and apically to the minute mass of embryonic cells at the tip. The entire arrangement comes to an end with the season, and a new set of mycorrhizae may be developed in the next season. The presence of the fungus is not necessary for the germination of pine seeds, and the plantlet can carry on normal development for two, perhaps three, seasons without mycorrhizae. Conditions adverse to the growth of the soil-fungus may result in seasons in which no mycorrhizae are formed with consequent impairment of the nutrition of adult trees.

Similar seasonal conditions have led to contradictory conclusions by observers, some of whom have examined individual plants with mycorrhizae,

while others have encountered individuals under conditions in which the cooperative arrangement was not completed due to adverse soil conditions.

Close and continuous engagement of the two symbionts, including the entire life of the seed-plant, is to be seen in some cases. The presence and action of compounds elaborated by the fungus may be necessary for the germination of the seeds and growth to maturity as exemplified by Corallorhiza. The mycelium in the cortex which keeps pace with the extension by growth of the rhizomatous stem sends hyphal branches *outwardly* into the soil simulating root hairs. The fungus forms a mycelium in the cortex of the internodes of Aplectrum which it enters through trichomes near the base, and the presence of the fungus is in transit toward the apex from which entrance in the roots of the next internode is made. Hyphal branches pass outwardly through the root hairs into the soil in this case. As has been previously described, unknown factors may cause unusual activity in the mycelium in its passage through the internodes with resultant development of coralloid branches. Numberless different types of engagement of the two symbionts might be cited, but the nutritive relations of the two plants concerned can be reliably determined only by a cytochemical analysis of the flow of material.

Genetical considerations

No conclusive evidence as to the manner in which characteristic morphogenic alterations, some of which are of wide scope, were initiated and became transmissible by seeds is available. Vegetative mutations in which underground stems (offsets) of Aplectrum respond to penetration of hyphae by the formation of mycorrhiza branches identical in general features with the underground coralloid stems of the nearly related Corallorhiza have been previously described (**6**). Such mutations occur numerously and in widely separated habitats but may be induced culturally and are not transmissible by seeds. The transformation is firmly fixed in Corallorhiza and the mutation affects the system of genes to such an extent that the capacity for the formation of roots has been lost entirely. Reduction of foliar surfaces and of chlorophyll, with accompanying modifications of the transpiratory mechanism, are very marked.

Attention is naturally directed to the minute seeds of these orchids and to the part which the symbiotic fungus may play in their germination. Cultural tests of a number of species of terrestrial orchids show that the seeds of some are activated only in the presence of the symbiotic fungus, others in water, and others in nutrient solutions to which some form of sugar, levulose, dextrose, or sucrose has been added (**1**).

In the case of *Corallorhiza innata,* which offers all of the morphological and physiological features of *C. maculata,* DOWNIE (**2**, **3**, **4**) found that "the seed of this orchid will not germinate in water nor in sugar solutions to which plant extracts have not been added," and it may safely assumed that *C. maculata* also requires the presence and action of substances derived from the fungus for the awakening of the embryo.

The minute seeds of this plant consist of a pear-shaped embryo about 0.02 mm. in length and a thickness of 0.01 mm. in the larger part, enclosed in a fragile transparent testa. This delicate structure would appear to be singularly vulnerable to environmental agencies, but such seeds have been seen to lie in moist humus for many months with comparatively few fatalities. It is in this condition that hyphae of the symbiotic fungus may be seen to enter the cells of the smaller end of the embryo and form coils or pelotons in the neighborhood of the nuclei, and some of the outer cells of the suspensor elongate to form absorbing trichomes similar to those of the adult rhizomes. Development of the embryo of *C. innata* was such a low rate that its length increased only eight times in nine months.

Germination of seeds of *C. maculata* showed a similar low rate. The contents of several pods placed in a layer of moist soil from the habitat in October, 1944, presented but little change until late in May, 1945; at this time a few embryos had begun enlargement as observed with a magnification of 80. Most of the testas were still intact; some hyphae penetrating the coats to the embryos were seen. The identity of the cytochemical processes of the orchid mycorrhizae with those of the pines may justifiably be extended to include accelerated absorption, respiration, and metabolism as described by ROUTIEN and DAWSON (9).

The uptake of oxygen in mycorrhizal roots was two to four times that of non-mycorrhizal members, with consequent increase of energy available for carrying nutrient substances across membranes. Anaerobic respiration was also increased with implied greater production of carbon dioxide and an increased supply of potential hydrogen ions.

It would be reasonable to attribute augmented possibilities of genetical modifications in young mycorrhizal protocorms with many metabolic processes being carried at unusual high levels. Compounds representing the main components of the cells are formed in the hyphae including a carbohydrate, glycogen, which non-diffusible, becomes so when phosphorylated and after translocation appears as glucose in cortical cells where synthesis of starch takes place.

The translocation of acid-soluble, mono-nucleotides to the cells of the embryo would disturb the balance in the cytoplasm with the desoxy-, highly-polymerized, ribose nucleotides of the chromosomes, while the introduction of any foreign proteins might have a disturbing effect. It is also to be said that the addition of phosphorous complexes which would enter into competition with those normally playing a part in the respiratory systems might be a factor in inducting genetical departures.

Whatever the nature of the cytochemical action, the genes responsible for the origination and production of roots in Corallorhiza have been cancelled or masked so that no rudiments of these organs are to be seen in any stage of the development of the vegetative axis. Reduction of the photosynthetic mechanism to a variable near the vanishing point has also occurred.

Concomitant with the losses or degradations mentioned, the overall system of metabolism has undergone adjustments. In addition to the reception of auxins, hormones, etc., from the associated fungus all of the materials entering into flower, fruit, and seed formation may be produced in the mycorrhizal rhizomes and translocated apically to the inflorescence as described by MACDOUGAL and REED (**7, 8**).

No evidence to the effect that the association of a fungus with its mycorrhizal partner is necessary for its continued existence is yet available. Neither has any determination of possible alterations been made. The results of cultural experiments with other microorganisms suggests the possibility that the nutritive conditions furnished by cortical cells might induce the development of specialized strains of the fungus not easily separable from the main mass of the mycelium.

Summary

1. The nutritive relations of associated plants may vary by minute gradations from pathogenic parasitism through symbiosis to effects purely mechanical and incidental.

2. The main components of protoplasm may be elaborated by the fungi of orchid and pine mycorrhizae and translocated to the cortex.

3. The reaction of cells of the higher plant entered by fungi is determined by the balance between the agencies which activate hydrogen and those which activate oxygen.

4. Decompensated respiration, especially in cells of the pericycle and endodermis, results in polymerization of the quinoids into gummy tannin masses, the presence of which forms a barrier to the extension of hyphae.

5. Technique of cytochemical analysis of nutritive relations is described.

6. The minute seeds of terrestrial orchids present many possibilities of genetical modification which might result from the action of environic factors and especially of substances received from associated fungi which enter the embryo at an early stage. Accelerated metabolism and the translocation of foreign nucleoproteids and of phosphorous complexes are of special significance. The principal mutations include loss of roots, reductions or total loss of photosynthetic mechanisms, and the acquisition of a capacity to mature seeds in the absence of photosynthesis.

R.F.D. 1, BOX 170
CARMEL, CALIFORNIA
CUTTER LABORATORY
BERKELEY, CALIFORNIA

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GROWTH STIMULATION AND PHOSPHORUS ABSORPTION OF MYCORRHIZAL AND NON-MYCORRHIZAL NORTHERN WHITE PINE AND DOUGLAS FIR SEEDLINGS IN RELATION TO FERTILIZER TREATMENT[1]

A. L. McComb and John E. Griffith

(WITH FOUR FIGURES)

Although there is no longer much question of the dependence of pines and many conifers on fungal symbionts forming mycorrhizae, there is considerable disagreement concerning the manner in which this symbiosis stimulates the growth of trees. A marked change and enlargement of the tree seedling root system follows mycorrhiza formation, and according to various authors the fungus partner makes available to the tree organic nitrogen (**6**), carbohydrates (**4, 11**), water (**1**), nutrients (**2, 4, 5, 10**), auxins and enzymes (**3, 4, 8**), and in addition destroys toxic soil compounds resulting from certain types of organic decomposition (**7, 8**).

In a recent paper one of the authors (**5**) described a case of the dependence of pines grown on prairie soil on mycorrhizal symbionts, showing the importance of phosphorus and suggesting that growth stimulation was due to increased respiratory activity associated with higher levels of phosphorus and with growth stimulators derived from the fungus. Routien and Dawson (**10**) have shown increased salt absorption associated with the heightened oxygen intake and carbonic acid production of mycorrhizal *Pinus echinata*. MacDougal and Dufrenoy (**4**) indicate that mycorrhizal fungi supply phosphorus and other nutrients, carbohydrates, auxins, and B vitamins and suggest that much of the increased activity of mycorrhizal roots is keyed to the metabolism of phosphorus.

This investigation was concerned with the growth and phosphorus absorption of two conifers on inoculated and uninoculated soil given several fertilizer treatments. Its primary object was to determine what combination of phosphorus application and/or soil inoculation produced best development and how phosphorus absorption was affected.

Experimental

This was a field experiment carried out in seed beds on O'Neill sandy loam at the State Forest Nursery, Ames, Iowa. Two adjacent beds were selected on soil which had never grown conifers before, and which previous experience had shown did not contain active mycorrhizal fungi. One bed was treated at the rate of one bushel per 400 sq. ft. with coniferous duff and humus known to contain active mycorrhizal formers. This bed and the other were divided into three replicates for each species and individual random-

[1] Journal Paper no. J–1308 of the Iowa Agricultural Experiment Station, Project no. 612.

ized plots treated with one of six phosphorus or phosphorus nitrogen and potassium combinations. The treatments were as follows:

	Pounds per acre of fertilizer in treatments						
	Treatments						
	1	2	3	4	5	6	7
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
20% superphosphate	.....	500	1000	1500	1000	1000	1000
Ammonium sulfate	.....	.....	.....	.....	150	300	150
Potassium sulfate	.....	.....	.....	.....	.....	.....	300

Part of each bed was seeded in June, 1940, to northern white pine (*Pinus strobus* L.) and the other part to Douglas fir (*Pseudotsuga taxifolia* (La Marck) Britt.). In May, 1941, the beds were thinned to reduce competition effects, and plots receiving nitrogen were refertilized with ammonium sulphate. During the fall of 1941 the seedlings were removed, twenty seedlings being selected mechanically from each plot for growth measurements and study of the root systems, the remainder dried and ground for analysis. Phosphorus was determined by igniting 1 gram ground tissue with $Mg(NO_3)_2$ and HNO_3, taking up residue in H_2SO_4, diluting, and determining colorimetrically as ammonium-phospho-molybdate.

Two-year-old white pine seedlings (fig. 1) made satisfactory growth on all plots except the uninoculated controls. There was no significant difference in weight or height among any of the inoculated or any of the uninoculated fertilized plots. Only the uninoculated, unfertilized controls (fig. 2) were significantly different from the others, and this difference corresponded to a much lower tissue phosphorus content and to a marked reduction in the number of short roots. No significant difference associated with nitrogen or potassium fertilization was noted.

Examination of the short roots of white pine showed three classes to be present (fig. 3): (1) hypertrophied with a macroscopically visible, external fungal mantle and with inter- and intra-cellular hyphae; (2) hypertrophied with a tight, compact, mantle, microscopically visible only on sectioning and with intercellular hyphae only; and (3) roots not hypertrophied nor mycorrhizal. All of the short roots of seedlings on the uninoculated, unfertilized plots were non-mycorrhizal (class 3). Short roots of seedlings from inoculated plots regardless of fertilizer treatment were of all three classes, while roots from uninoculated fertilized plots were chiefly of classes 2 and 3. The most significant points in the white pine data appear to be that phosphorus fertilization induced the formation of mycorrhizae in uninoculated plots, and that without either inoculation or fertilization, both of which resulted in mycorrhizae, the seedlings eventually died.

Douglas fir seedlings (fig. 4) made satisfactory growth on inoculated plots only. On uninoculated plots growth increased with increase of phos-

phorus application, but in no case was fertilization equivalent to inoculation. Seedlings from uninoculated, unfertilized plots were dying at the end of the second growing season. Nitrogen and nitrogen and potassium, both in

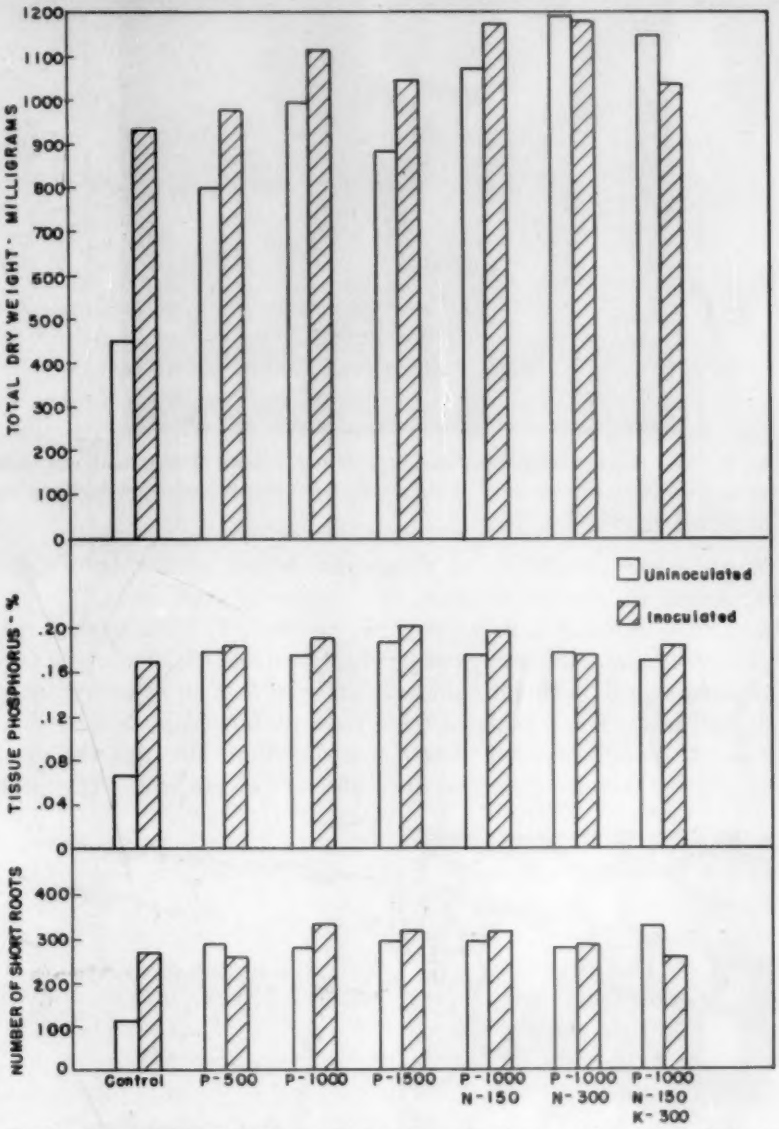

FIG.1. Dry weight, percentage of phosphorus in tissue, and number of short roots of northern white pine on inoculated and uninoculated O'Neill soil treated with various fertilizers.

combination with phosphorus, caused no significant growth increase over that obtained using phosphorus alone. The most significant points in these data are the marked superiority in growth of seedlings from inoculated soil

FIG. 2. Two-year-old white pine seedlings on unfertilized O'Neill soil. Inoculated (above) and uninoculated (below). Note small, fortuitously inoculated seedling clump in left center of uninoculated plot.

and the three-way parallelism on uninoculated plots among dry weight or height, phosphorus content of tissue, and number of short roots.

Short roots of Douglas fir were of two classes: (1) those hypertrophied but with no fungal mantle externally visible with the microscope and (2) non-hypertrophied. Sectioning showed that roots lacking hypertrophy were non-mycorrhizal. Hypertrophied roots from inoculated plots were mycorrhizal and from uninoculated plots non-mycorrhizal, although the number of roots studied was insufficient to draw absolute conclusions. The number

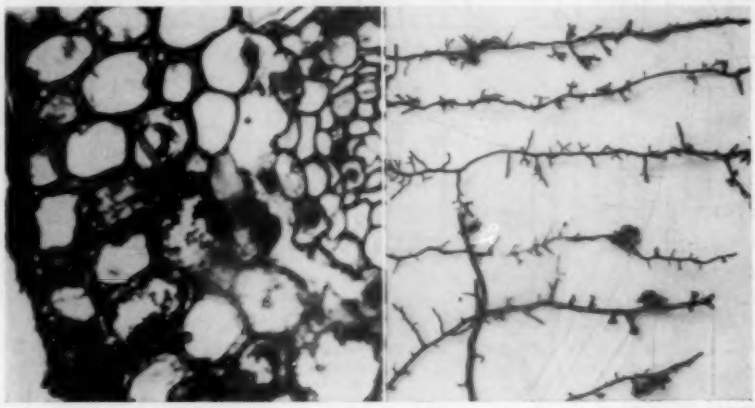

FIG. 3. White pine mycorrhizae. Left, section showing inter- and intra-cellular hyphae (325×); right, root branches with mycorrhizae. Coralloid clumps below are class 1; other hypertrophied shoot roots are class 2.

of hypertrophied short roots on uninoculated plots increased with phosphorus application from 7 per average seedling in the control to 31 for the 1500-pound superphosphate application. Thus, as contrasted to the white

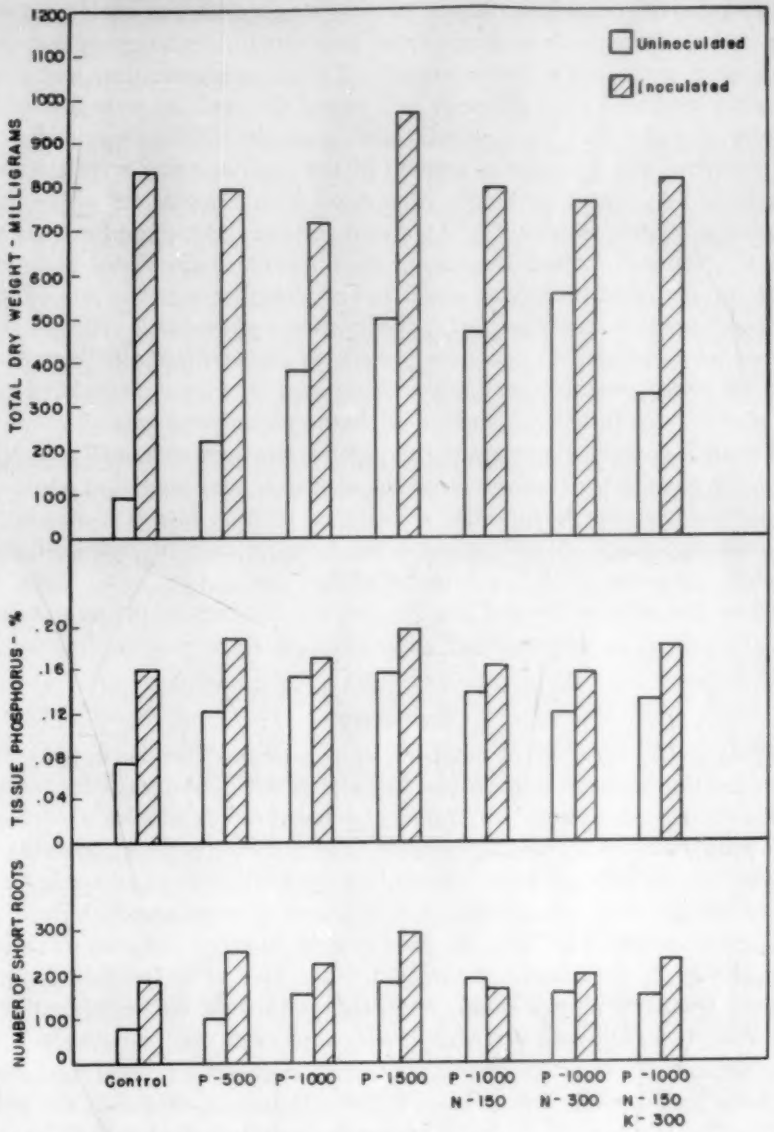

FIG. 4. Dry weight, percentage of phosphorus in tissue, and number of short roots of Douglas fir on inoculated and uninoculated O'Neill soil treated with various fertilizers.

pine, phosphorus fertilization of Douglas fir on uninoculated soil did not result in mycorrhizae formation even though some root stimulation took place.

Considering the data for both species the growth stimulation due to straight soil inoculation followed by mycorrhizae formation is outstanding. The formation of mycorrhizae on fertilized, uninoculated plots of white pine and the difference in structure of these mycorrhizae, as contrasted with some of those forming on seedlings from inoculated plots, suggests that there was present in the soil a fungus capable of forming mycorrhizae and stimulating the seedlings when either or both it and the seedling were first stimulated by phosphorus. No such relationship obtained with Douglas fir.

In general, the phosphorus content of the seedlings was correlated with growth. This is most strikingly illustrated with Douglas fir where tissue phosphorus concentrations of 0.16 per cent. appeared adequate for maximum growth. Non-mycorrhizal Douglas fir seedlings on uninoculated plots grew faster and absorbed increasing quantities of phosphorus as the rate of phosphorus application increased, *but, although tissue phosphorus concentrations reached levels producing adequate growth of mycorrhizal seedlings on inoculated plots, growth of non-mycorrhizal seedlings on uninoculated plots was significantly inferior.* Since total tissue phosphorus was adequate for good growth and since no significant response has been obtained with N, K, or Ca, the obvious conclusion is that the seedlings were receiving additional stimulators from the mycorrhizal association. These data all indicate that the most important rôle of mycorrhizal fungi is that of stimulating the metabolic processes of the roots and that this stimulation, as has been suggested by the authors (**5**) and MacDougal and Dufrenoy (**4**), is associated with the rôle of phosphorus and other stimulators in root respiration and metabolism.

Summary

Two-year-old seedlings of northern white pine and Douglas fir made very satisfactory growth on O'Neill soil inoculated with coniferous humus containing mycorrhizal fungi. On uninoculated soil fertilized with phosphorus, white pine seedlings formed mycorrhizae and made satisfactory growth; but Douglas fir seedlings, although responding moderately to phosphorus fertilization, did not form mycorrhizae nor maintain normal growth rates. Excepting uninoculated Douglas fir, good growth in every instance was associated with high phosphorus absorption. The fact that Douglas fir made poorer growth on uninoculated, fertilized plots than on inoculated plots, even though seedling phosphorus levels were high, and no response was being obtained from nitrogen and potassium, suggests a mycorrhizal stimulus above that directly due to phosphorus. It is suggested that the stimulating effect of mycorrhizal fungi on conifer seedlings is due to heightened metabolism, associated in this instance with transfer of phosphorus and growth stimulators from fungus to seedling.

Forestry Department
Iowa State College
Ames, Iowa

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EFFECT OF VARIOUS OXYGEN AND CARBON DIOXIDE CONCENTRATIONS ON COTTON ROOT DEVELOPMENT[1]

O. A. Leonard and J. A. Pinckard

(with nine figures)

Introduction

Under field conditions, the concentrations of oxygen and carbon dioxide at the surface of cotton roots are thought to be extremely variable. Gross analyses made by Leonard (10) show the percentage of oxygen in the soil air under cotton to vary from 0 to 21 for Houston clay, from 10 to 21 for Sarpy fine sandy loam, and from 18 to 21 per cent. for Ruston sandy loam. While these values only approximate effective concentrations at root-gas interfaces, they do indicate in a relative way the conditions of aeration within different soil types. Although the differences in behavior of cotton on these different types of soil may be attributed to differences of particle size, fertility, water movement, and numerous other variables, the rôle of aeration appears to be quite important. Unpublished data of the present writers indicate that aeration and mechanical composition of the soil may prove to be an important factor in the distribution of the Fusarium wilt disease of cotton. The importance of soil aeration as it applies to the culture of plants in general is well appreciated, although many of the details are yet to be investigated.

The purpose of the present study was to determine the effect of carbon dioxide and oxygen mixtures on the growth and development of cotton roots in nutrient solutions. The study seemed necessary in order to obtain data for the proper interpretation of the behavior of cotton on different soil types with and without the presence of the Fusarium wilt disease.

Methods and materials

The general plan of the apparatus employed for subjecting the roots of seedling cotton plants to stated concentrations of oxygen and carbon dioxide alone and in mixtures with nitrogen is illustrated in figure 1. By means of a siphon arrangement, the gas in container B was forced through a nutrient solution in which the plants were supported. Although only one culture tube and one plant is illustrated in figure 1, two tubes were used, each containing two plants. A battery of several such units was used, each provided with a different concentration of gas. The entire equipment was operated before a large laboratory window or in the greenhouse. Supplementary illumination was provided by a fluorescent lamp which was operated continuously. In some experiments (not reported) no supplementary illumination was used; in others, artificial light supplemented daylight sufficiently

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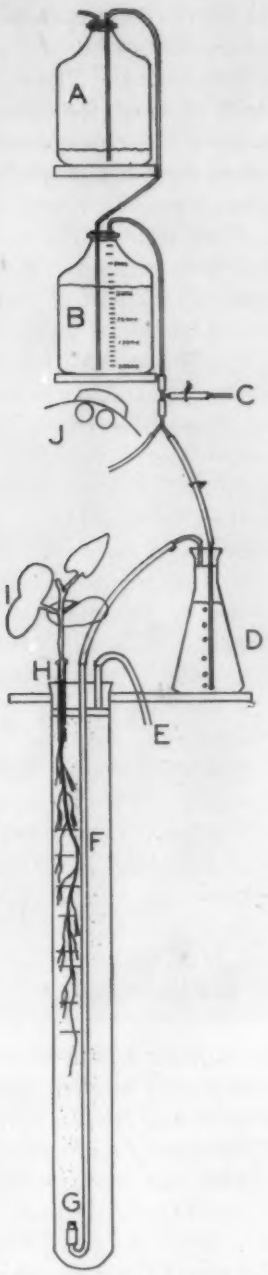

FIG. 1. A diagrammatic view of the apparatus used in aeration experiments. A, five-gallon bottle; B, five-gallon bottle calibrated to hold 20 liters of air at atmospheric pressure; C, air inlet for making gas mixtures; D, Erlenmeyer flask holding reserve nutrient solution; E, gas escape tube; F, culture tube; G, gas outlet; H, glass tube in which cotton plant was inserted; I, cotton plant; J, fluorescent light.

to make a photoperiod of 15 hours. The relationship between the percentage of oxygen and carbon dioxide and cotton root growth was similar to that obtained with continuous illumination. The culture tubes were placed in a darkened air- or water-bath in which the temperature was maintained at approximately 30° C. in some experiments and 28° C. in others. Air temperatures about the shoots were not controlled.

Compressed cylinder gases were either used directly or further purified by appropriate methods. They were introduced into the system through opening C and were utilized from container B (fig. 1). Approximately 40 liters of gas was passed through each pair of culture tubes in 24 hours. To prepare a given concentration of mixed gases, distilled water was forced from calibrated container B. The composition of the mixtures were determined at frequent intervals by a portable gas analysis apparatus. Oxygen concentrations were easily prepared accurately, although carbon dioxide concentrations frequently varied over a range not exceeding 10 per cent. of the required concentration. Dissolved oxygen in the culture solution was determined by the Winkler method (**1**).

A modified Knop's solution of approximately the same composition shown by LOOMIS and SHULL (**12**) was used in the culture tubes. In tests in which nitrogen was supplied in the ammonium form, ammonium chloride and calcium chloride replaced an equivalent quantity of calcium nitrate. The initial pH of the nutrient solution was adjusted at 5.4 to 5.6 using a weak solution of potassium hydroxide, the exact pH varying somewhat with different experiments. The nutrient solutions containing nitrates usually shifted to a pH of about 6.0 and the solutions containing ammonia to a pH of about 4.8 before being changed. If the solutions containing ammonia become too acid (pH of 4.0 or less) root growth ceased. The pH of solutions receiving high concentrations of carbon dioxide were stabilized by using a buffer composed of potassium acid phosphate and potassium hydroxide. The solutions were prepared from distilled water and most of them were changed at intervals of not more than three or four days. Solutions saturated with pure gases were not changed during the course of an experiment.

Cotton seeds of the Stoneville 2B variety were acid delinted, dusted with a mercurial disinfectant, and placed between moist paper towels for germination. If no seed disinfectant was used, a low yield of healthy seedlings resulted, even though all glassware, solutions, and apparatus were boiled or autoclaved before use. After the seeds germinated, the seed coats were removed with sterile forceps and the seedlings supported in tap water on a paraffin-impregnated cloth. After the cotyledons were expanded, healthy seedlings were selected, and the radicals were inserted through short glass tubes held in no. 7 rubber stoppers. A paraffin-petroleum-cotton-lint sealing compound held the seedlings in place and prevented exchange of gases through the openings after the stoppers were placed in the culture tubes. Air was passed through the culture solution for two or three days to permit

the seedlings to become established. The air or gas was released through a sintered glass plate in the bottom of the 24-inch culture tubes so as to break up the gas bubbles.

Growth of the seedling roots was determined by measuring the length of the tap root at 24-hour intervals. The experiments were terminated 14 to 16 days after the gases were first introduced. Notes were made on the

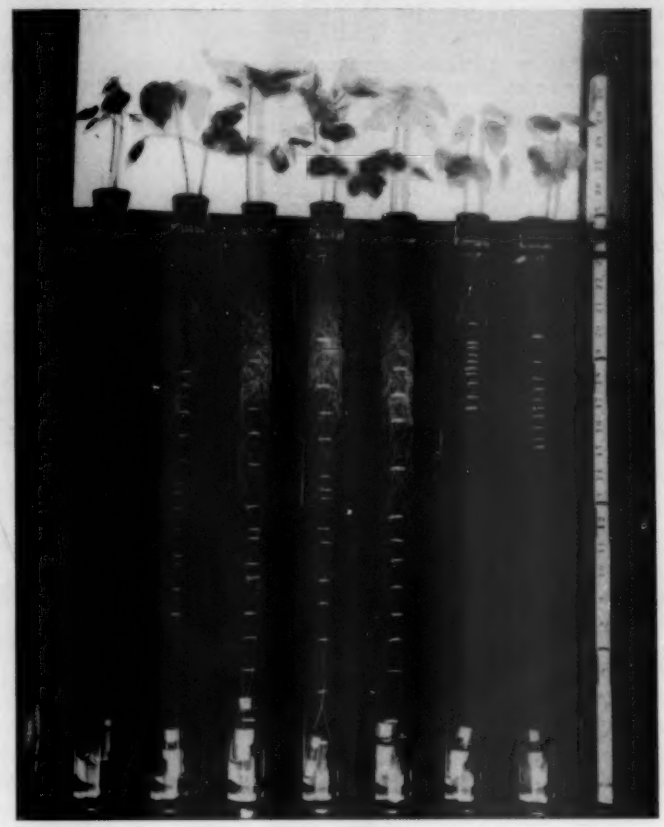

FIG. 2. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 13). Marks on the tubes represent tap-root elongation at 24-hour intervals. The gas mixtures from left to right were (percentages):

O_2	0	5	10	15	21	90	100
CO_2	10	10	10	10	10	10	0

appearance of the seedlings, and the fresh and dry weights of the shoots and roots were recorded.

A total of 20 separate experiments were conducted over a period of one and one-half years; although only seven of these experiments are actually referred to in the text, the results from the other experiments were similar to the ones reported.

Results

Oxygen requirements

The optimum oxygen concentration for cotton root development was thought to depend upon a certain range of carbon dioxide concentration. To determine this range, oxygen, carbon dioxide, and nitrogen were combined in the proper proportion to form the desired mixtures. Oxygen was

TABLE I

Appearance of 14-day-old cotton seedlings grown in liquid cultures saturated with carbon dioxide and oxygen at stated concentrations and with amount of dissolved oxygen in the cultures. Results from several experiments summarized

Gases used		Dissolved oxygen in cultures*		Appearance of tops	Appearance of roots
CO_2	O_2	With plants	Without plants		
%	%	*p.p.m.*	*p.p.m.*		
None	None	0.0	0.0	Little plants, small green leaves	Roots healthy, no growth. Branch roots developed if transferred to water saturated with air
10	None	0.0	0.0	Little plants, dark green leaves	Do.
10	0.5	Trace	0.1	Do.	Do.
10	1.0	0.3	0.4	Little plants, green leaves	Roots healthy, trace of growth
10	1.5	0.5	0.6	Medium plants, green leaves	Roots healthy, thick, some growth
10	2.5	0.8	1.0	Large plants, green leaves	Roots healthy, medium thick, good growth
10	5.0	1.8	2.0	Do.	Roots healthy, thin, very good growth
10	7.5	2.7	3.0	Do.	Do.
10	10.0	3.6	3.8	Do.	Do.
10	15.0	5.4	5.8	Do.	Do.
10	21.0	7.0	.....	Do.	Do.
10	90.0	33.0	.....	Medium plants, green leaves	Roots frequently unhealthy and parasitized, poor growth. Healthy roots produced if transferred to water saturated with air
None	100.0	37.0	.....	Do.	Do.

* Temperature of 28° C.

held constant at 21 per cent. while carbon dioxide and nitrogen were varied. Considerable root growth occurred up to 30 per cent. carbon dioxide while vegetative growth appeared to be excellent and unaffected over the same range. As a result of these tests, 10 per cent. carbon dioxide was selected as a constant with which to compare different concentrations of oxygen.

The apparent optimum concentration of oxygen for seedling root elongation, with carbon dioxide held constant at 10 per cent. was found to range

between 7.5 and 21 per cent. under the conditions described. Figure 2 illustrates a typical experiment in this series, and table I summarizes the results of all oxygen concentrations investigated. Figure 3 illustrates the variation occurring in three selected experiments.

Daily elongation of the tap roots is graphically illustrated in figure 3. During the first two weeks of seedling growth, tap-root elongation appeared to progress at a uniformly high rate. The greatest elongation for any one day was 67 mm.; it occurred at 21 per cent. oxygen and 10 per cent. carbon dioxide. Variation in root temperatures slightly above 30° C. sharply restricted the daily rate of tap-root elongation. Any prolonged reduction in intensity of illumination also limited root elongation, but the effect was not apparent for two or three days. Experiment 15 was conducted at light intensities somewhat lower than that of Experiment 17 (fig. 3). Although

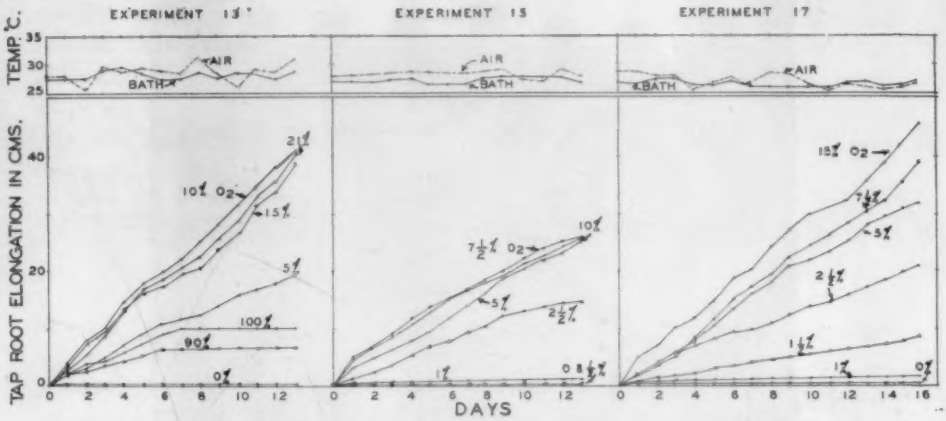

FIG. 3. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots. Carbon dioxide was held constant at 10 per cent., except at zero per cent. oxygen in Experiments 13 and 17 and at 100 per cent. oxygen in Experiment 13.

two plants were grown in the same tube and duplicate tubes used, the daily rate of elongation for each plant varied widely at times. No relation could be observed between stage of leaf development or leaf area and tap-root elongation.

The minimum concentration of oxygen tolerated by roots appeared to lie below one-half per cent. (figs. 2, 4) and zero per cent. (fig. 5). No root growth occurred under these conditions. The roots remained white and did not appear to be injured by anaerobic conditions. If air was substituted for the mixture containing zero oxygen, lateral roots were promptly initiated, and some of the old branch roots began to grow again. The tops of the plants were a darker green (when carbon dioxide was held at 10 per cent.) than those of plants receiving higher concentrations of oxygen.

At maximum concentrations of oxygen (90 and 100 per cent.), both root and top growth were restricted (fig. 2), but the color of the leaves was not affected as at low oxygen concentrations. If air replaced the gas mixtures

of high oxygen concentrations, lateral root development was initiated, indicating that the pericycle was not permanently damaged. Microorganisms attacked the roots vigorously at high oxygen concentrations. Although

FIG. 4. The effect of various concentrations of oxygen on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 5). Although the gas mixtures were the same as those used in figure 2, the bubbles were not broken up with a sintered glass plate, and the solutions were not kept saturated with oxygen. The "effective" percentages of oxygen were calculated from the determinations of dissolved oxygen. Marks on the tubes represent tap root elongation at 24-hour intervals. The calculated percentages of oxygen and carbon dioxide from left to right were:

O_2	0	2½	5	10	15	85	95
CO_2	10	10	10	10	10	10	0

care was exercised in preventing microbial contamination, it was difficult to prevent the roots from being decomposed at the high oxygen concentrations.

If the concentration of oxygen was reduced or if air was substituted, decomposition ceased, and lateral roots developed. Species of Fusaria appeared to be dominant. The organisms present were non-parasitic at lower oxygen levels. This interesting observation deserves further study.

The amount of dissolved oxygen in the nutrient solutions receiving the various percentages of oxygen-gas mixtures is given in table I as parts per million. The exact amount of dissolved oxygen varied with temperature. Estimations of dissolved oxygen enabled us to be certain that the rate of gas evolution through the nutrient solutions was sufficient to approximately saturate the solution. It also showed that solutions with no plants growing in them were consistently, though only slightly, higher in dissolved oxygen than solutions containing growing plants. When the rate of gas evolution through the solutions was reduced, the roots absorbed oxygen, and the actual concentration of oxygen in the cultural medium was less than the calculated amount introduced. Figure 4 illustrates the results of a typical experiment of this type. The actual concentrations of oxygen in the solutions was determined by analysis. When 10 per cent. carbon dioxide was used in the mixture, the limiting oxygen factor was well illustrated by reduced root and shoot growth at both low and high concentrations.

Effect of form of nitrogen in the nutrient solution on oxygen requirement

The effects of nitrate and ammonium nitrogen on root growth were compared at all oxygen concentrations from zero to 100 per cent. The chief interest was in the effect of nitrate and ammonium nitrogen on cotton root growth at low percentages of oxygen. The results from one such experiment are shown in figures 5 and 6 and summarized in figure 7.

Tap-root elongation was similar with both nitrate and ammonium nitrogen at all concentrations of oxygen. Roots subjected to zero per cent. oxygen remained white and appeared healthy with both nitrate and ammonium nitrogen. Shoot growth seemed greater with nitrate than with ammonium nitrogen, regardless of the oxygen concentration.

Up to 15 per cent. oxygen content tap-root elongation increased, while the weight of the root system was near or at its maximum at two and one-half per cent. oxygen. The lack of correlation between tap-root elongation and increase in weight of the whole root system was caused, at least in part, by the fact that the roots which developed at the lower oxygen concentrations were thick, while those produced at higher percentages of oxygen were slender; thus a given length of root at two and one-half per cent. oxygen weighed more than the same length of root produced at higher oxygen concentrations.

Cotton seedlings growing in solutions containing nitrates accumulated more nitrogen than did those growing in solutions containing ammonia. The extra nitrogen absorbed (in nitrate form) from the solutions containing nitrate over those containing ammonia seemed to accumulate as nitrate

nitrogen within the plants, since the percentages of organic nitrogen in the whole plant was about the same in both the nitrate and ammonia series. The total absorption of nitrogen (in nitrate or ammonium form) increased with oxygen increase up to about two and one-half per cent. The differences in

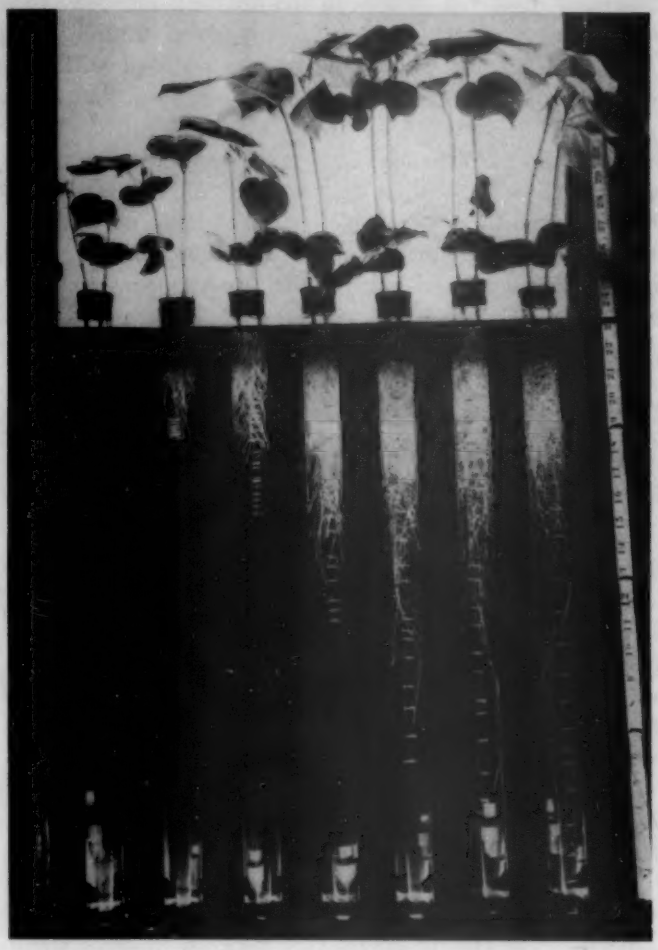

FIG. 5. The effect of nitrate nitrogen on the daily elongation of cotton tap roots and appearance of the shoots at various concentrations of oxygen (Experiment 17). Marks on the tubes represent tap-root elongation at intervals of 24 hours. The gas mixtures from left to right were (percentages):

O_2	0	1	1½	2½	5	7½	15
CO_2	0	10	10	10	10	10	10

total uptake of nitrogen were largely caused by differences in plant size, rather than by oxygen concentrations. Nevertheless, the percentage of nitrate nitrogen in the plant increased with oxygen concentration and suggests better absorption of nitrate nitrogen at high than at low oxygen concentrations: The percentage composition is based on an analysis of the whole

plant, and the results may be affected by differences in the relative amounts of roots, stems, and leaves.

In order to study the absorption of nitrate and ammonium nitrogen at different concentrations of oxygen, the nutrient solutions were periodically analyzed. Results with the ammonia series suggested that more ammonium

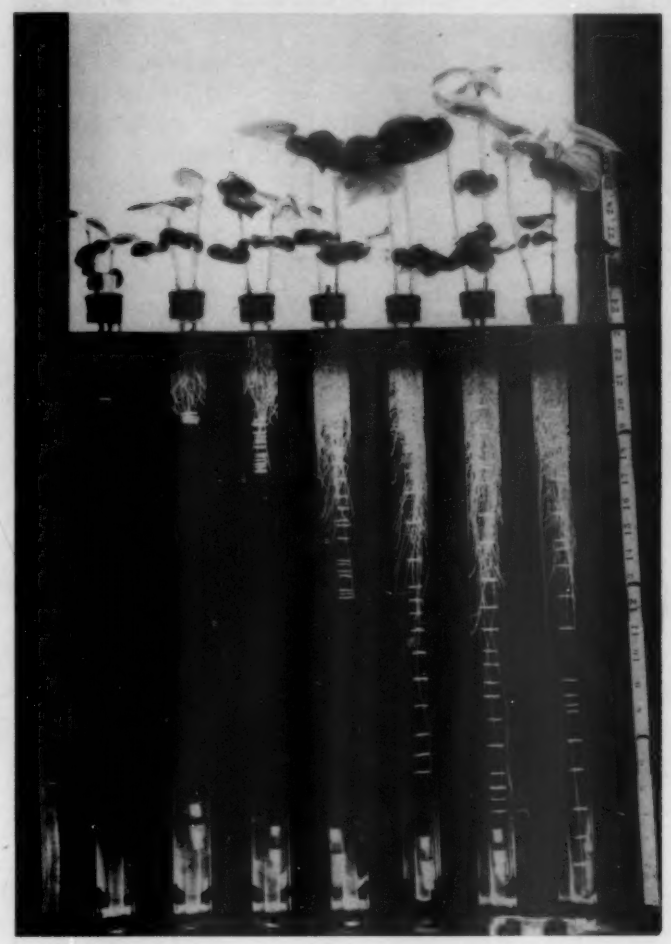

FIG. 6. The effect of ammonium nitrogen on the daily elongation of cotton tap roots and appearance of the shoots at various concentrations of oxygen (Experiment 17). Marks on the tubes represent tap-root elongation at intervals of 24 hours. The gas mixtures from left to right were (percentages):

O_2	0	1	1½	2½	5	7½	15
CO_2	0	10	10	10	10	10	10

nitrogen was absorbed at high than at low concentrations of oxygen. The results with the nitrate series indicated the greatest loss of nitrate from nutrient solutions occurred at zero per cent. oxygen. Since a chemical analysis of the plant did not reveal any comparable accumulation of nitrogen

within the plant, it was concluded that some nitrate nitrogen was lost anaerobically from the nutrient solution.

Effect of carbon dioxide on cotton root growth

The influence of various concentrations of carbon dioxide on the daily elongation of the cotton tap root is shown in figures 8 and 9. Table II

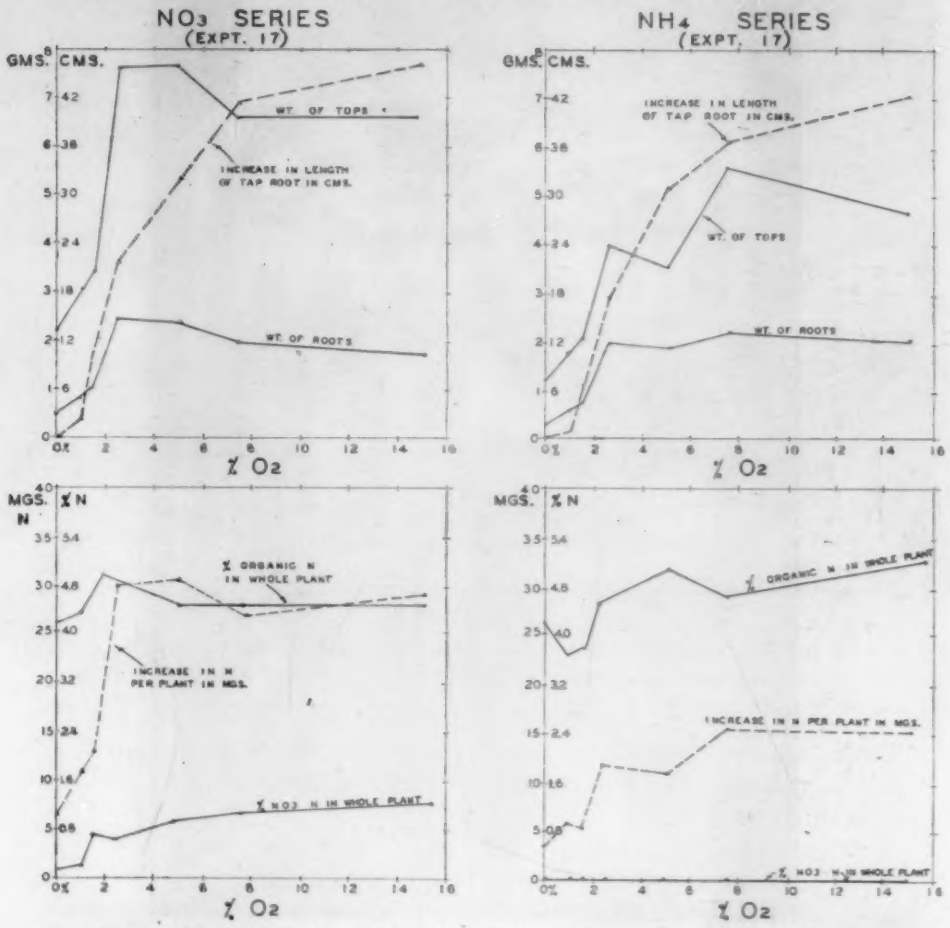

Fig. 7. The influence of various concentrations of oxygen and forms of nitrogen on the total elongation of the tap root, fresh weight of roots and tops, total absorption of nitrogen, and percentage of organic and nitrate nitrogen in seedling cotton plants.

summarizes the results obtained from various experiments in which carbon dioxide was varied. The results of the three experiments shown in figure 8 indicate that a concentration of 60 per cent. carbon dioxide prevented all cotton root growth. Concentrations of carbon dioxide higher than 60 per cent. had the same influence on root growth and appearance of the tops as did 60 per cent. carbon dioxide. The higher concentrations of carbon

dioxide resulted in wilting of the tops, especially when the cotton was in direct sunlight and when the air temperature was above 30° C. Wilting did not occur at the lower concentrations of carbon dioxide, with the exception of occasional wilting at 45 per cent. carbon dioxide.

The pH was controlled by a potassium phosphate buffer and potassium hydroxide. The pH of even the 100 per cent. carbon dioxide solution usually did not shift much below 4.9. Evidently the pH was adequately controlled, even when pure carbon dioxide was bubbled through the nutrient solution. In preliminary tests when the phosphate buffer was not used, pure carbon dioxide produced a pH of 3.6 in the nutrient solution.

Concentrations of carbon dioxide up to 15 per cent. resulted in the production of long, slender roots. Concentrations of 30 to 45 per cent. carbon

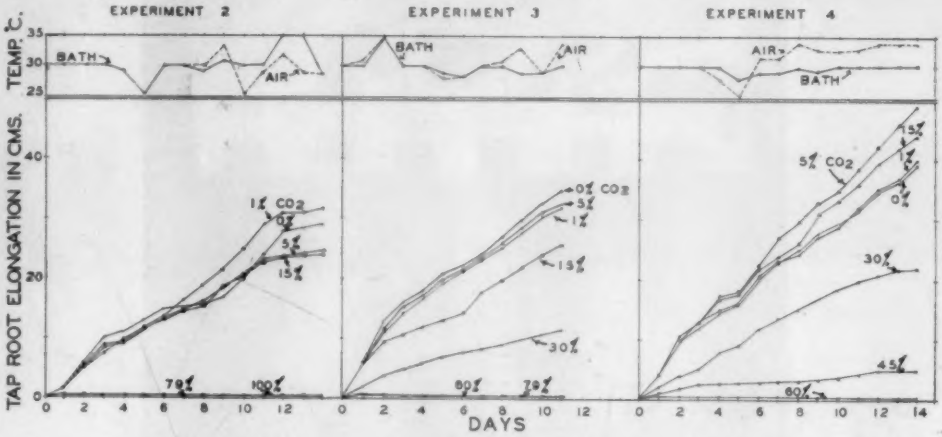

FIG. 8. The effect of various concentrations of carbon dioxide on the daily elongation of cotton tap roots. Oxygen was held constant at 21 per cent., except at 100 per cent. carbon dioxide.

dioxide resulted in the formation of somewhat short, thick roots. Sixty per cent. carbon dioxide, and higher, prevented growth either in length or in thickness of roots.

When 60 per cent. carbon dioxide was replaced by air (21 per cent. oxygen and traces of carbon dioxide) branch roots developed. These results indicated that the pericycle was not destroyed by the high concentrations of carbon dioxide. The tap root was never observed to elongate further, after the introduction of air.

Low concentrations and complete absence of carbon dioxide resulted in no apparent inhibition of root growth. All traces of carbon dioxide were removed by bubbling the gas mixture through gas wash bottles containing potassium hydroxide.

When the temperature of the bath was 35° C. or higher for 24 hours (fig. 8, Experiment 2), root elongation almost ceased at all concentrations, although roots in five to 15 per cent. carbon dioxide were affected most

promptly. The daily elongation of roots growing in the same environment varied for no apparent reason.

Discussion and conclusions

Cotton tap root elongation was entirely inhibited by gas mixtures containing one-half per cent. of oxygen or less. The roots remained white and apparently healthy under the above conditions for at least two weeks. If

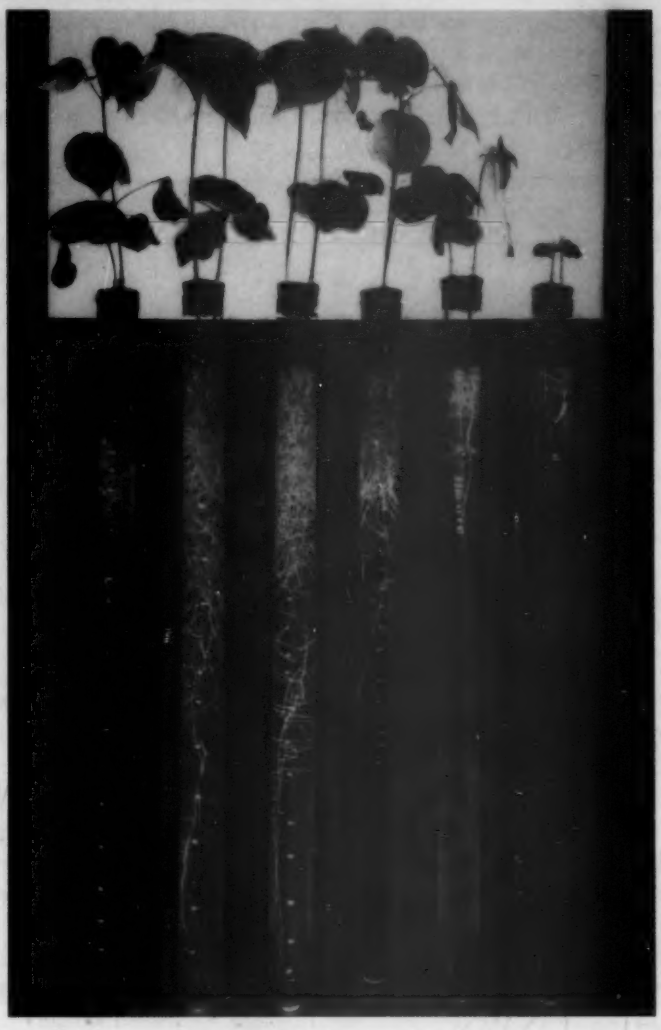

FIG. 9. The effect of various concentrations of carbon dioxide on the daily elongation of cotton tap roots and the appearance of the tops (Experiment 4). Marks on the tubes represent tap-root elongation at 24-hour intervals. The gas mixtures from left to right were (percentages):

CO_2	0	5	15	30	45	60
O_2	21	21	21	21	21	21

air containing 21 per cent. oxygen replaced the above gas mixtures, normal branch roots developed, and sometimes the branch roots began to grow again. Evidently, even very drastic reductions in oxygen around cotton roots, with either zero or 10 per cent. carbon dioxide, was not permanently harmful. The roots continued to absorb both nitrate and ammonium nitrogen, although not as well as cotton roots exposed to optimum concentrations of oxygen. The results suggest that the influence of extremely poor aeration of soil on the death of cotton roots is indirect. BALLS (2) observed that cotton roots apparently became asphyxiated when the water table rose. LEONARD (10) observed that when the soil was water-logged and very low

TABLE II

APPEARANCE OF 14-DAY-OLD COTTON SEEDLINGS GROWN IN LIQUID CULTURES SATURATED WITH OXYGEN AND CARBON DIOXIDE AT STATED CONCENTRATIONS. RESULTS FROM SEVERAL EXPERIMENTS SUMMARIZED

GASES USED		APPEARANCE OF TOPS	APPEARANCE OF ROOTS
O_2	CO_2		
%	%		
21	None	Leaves green, top growth good	Roots thin, very good growth
21	1.0	Do.	Do.
21	5.0	Do.	Do.
21	15.0	Do.	Do.
21	30.0	Do.	Root growth reduced, somewhat thick
21	45.0	Leaves green, reduced top growth	Root growth greatly reduced, thick
21	60.0	Leaves dark green, very little top growth, tending to wilt in sunshine	No root growth. Branch roots developed if transferred to nutrient solution saturated with air
21	79.0	Do.	Do.
None	100.0	Do.	Do.

in oxygen cotton tap roots were short and the tips dead. The influence of poor aeration on cotton roots might easily have been indirect by influencing the susceptibility of the roots to minor parasites.

The studies of VLAMIS and DAVIS (16) indicate that the oxygen requirement of excised roots of tomato, barley, and rice were similar, and yet the three plants reacted differently to anaerobic conditions. The evidence suggests that the reaction of the above plants to anaerobic conditions was related to their capacity to translocate oxygen from the stems to the roots. The capacity of cotton roots to remain alive and function well under anaerobic conditions is also probably related to its capacity to translocate oxygen from the shoots to the roots.

CONWAY (6), using *Cladium mariscus,* found a high oxygen content in intact roots but low oxygen in excised roots under similar conditions. The results suggest that oxygen is translocated to the roots. Differences in the

ability of plants to move oxygen to the roots may in part determine their behavior under anaerobic conditions.

Under anaerobic conditions, short branch roots were produced near the base of the hypocotyl. The tissue was bulky and contained some chlorophyll. Probably sufficient oxygen was present within the tissue of the hypocotyl, either from translocation or from photosynthesis, to enable root initiation.

The minimum concentration of oxygen for cotton tap root elongation was not influenced by the form in which nitrogen was supplied (that is, as nitrate or ammonium nitrogen). The results of VLAMIS and DAVIS (**16**) likewise indicate that nitrate nitrogen is not superior to ammonium nitrogen for either growth or potassium absorption. SHIVE (**15**), working with soybeans, found that the greatest absorption of nitrate nitrogen occurred from nutrient solutions containing no oxygen, and absorption of nitrate decreased as oxygen in the nutrient solutions increased. The absorption of ammonia, on the other hand, was the reverse of the absorption of nitrate. The results were explained as indicating that nitrates were supplying oxygen to the roots under anaerobic conditions.

Nitrate nitrogen tended to accumulate in cotton seedlings as the percentage of oxygen in the gas mixtures was increased. In view of the observation that the percentages of organic nitrogen in the whole plants were about the same with different percentages of oxygen, the accumulation of nitrate may signify differences in rates of nitrate absorption. Appreciable accumulation of nitrogen, however, took place even under anaerobic conditions.

PEPKOWITZ and SHIVE (**14**), working on tomatoes and soybeans, found that the absorption of calcium, phosphorous, and potassium was least at zero parts per million oxygen and rose to an optimum at higher oxygen concentrations; nevertheless, appreciable absorption still occurred, even with the zero oxygen treatment.

VLAMIS and DAVIS (**16**) found that the accumulation curves for bromide and potassium with different percentages of oxygen were about the same for excised roots of tomato, barley, and rice. A loss of potassium occurred in the absence of oxygen. The accumulation curve for intact roots was quite different from the above. Intact rice roots (shoots attached) in the absence of oxygen accumulated appreciable quantities of bromide; those of barley less; and those of tomatoes almost none.

The lower limit of oxygen necessary for root growth varies somewhat with temperature. According to CANNON and FREE (**4**) cotton root growth would occur at concentrations of oxygen of slightly less than one per cent., if the temperature was 17° C., but a concentration of over one per cent. would be necessary if the temperature was 30° C. A small amount of cotton root growth occurred at 28° C. with one per cent. oxygen.

In the present investigation the optimum concentration of oxygen for cotton tap root elongation, as well as cotton root growth, was between seven and one-half and 21 per cent. oxygen, although five per cent. oxygen was only slightly inferior. GILBERT and SHIVE (**7**) reported that the optimum

concentration of oxygen necessary for the production of green matter in soybeans was about six p.p.m.; in tomatoes, 16 p.p.m.; and in oats, four p.p.m.; or approximately equivalent to solutions in equilibrium with 15, 44, and 10 per cent. oxygen, respectively. BOYNTON and COMPTON (**3**) reported that oxygen concentrations of 15 per cent. or lower resulted in markedly poorer root and top growth of peach, apple, and prune trees growing in nutrient solutions than did 21 per cent. oxygen.

Excessive concentrations of oxygen resulted in reduced cotton root growth. Concentrations of either 90 or 100 per cent. oxygen gave similar results. Besides reducing the daily root growth, high concentrations of oxygen resulted in the roots becoming parasitized by what appeared to be chytrids in some cases and by species of Fusaria in others.

Root and shoot growth were reduced at oxygen concentrations above the optimum for root elongation. The leaves, however, appeared normal at the high oxygen concentrations, whether nitrate or ammonium nitrogen was used. GILBERT and SHIVE (**7**), working with soybeans, found that oxygen values of eight and 16 p.p.m. (equivalent to solutions in equilibrium with 22 and 44 per cent. oxygen) resulted in chlorotic leaves if nitrate nitrogen was present in the nutrient solution, but not if ammonium nitrogen was used. They referred to the chlorotic condition as one of "oxygen toxicity."

Excessive concentrations of carbon dioxide, as well as low concentrations of oxygen, are considered to influence the growth of plant roots in poorly aerated soils. Concentrations of 60 per cent. carbon dioxide, and above, inhibited all cotton root growth even though oxygen was held at an optimum concentration. The pH, likewise, was held constant in these experiments. Evidently, the influence of high concentrations of carbon dioxide on root growth was not caused by a deficiency of oxygen nor by an excessively acid condition of the nutrient solution. If air containing 21 per cent. oxygen replaced the inhibitory concentrations of carbon dioxide, normal branch roots were formed. Evidently, high concentrations of carbon dioxide did not kill the cells of the pericycle. Top growth was very poor when high concentrations of carbon dioxide were used. When the plants were in direct sunlight and the air temperature was 30° C., or higher, the tops wilted.

NOYES (**13**) passed carbon dioxide through soil in which corn and tomato plants were growing, causing the plants to wilt and growth to stop. CANNON and FREE (**4**) studied the influence of different concentrations of carbon dioxide on the root growth of a number of plants. They found that high carbon dioxide concentrations inhibited the growth of all plants if the periods of exposure were sufficiently long, but that the sensitivity of different plants to different concentrations of carbon dioxide was not the same. Root growth of *Krameria canescens* ceased if carbon dioxide was 25 per cent. and if the temperature was 30° C.; growth did not cease if the temperature was 20° C. and root growth continued even at 75 per cent. carbon dioxide. Although 25 to 75 per cent. carbon dioxide prevented root growth of *Covillea tridentata*, growth could again occur if the above mixture were replaced by air.

VLAMIS and DAVIS (16) have shown that pure carbon dioxide prevented the accumulation of bromide by both excised and intact roots of barley, tomato, and rice; it also resulted in wilting and cessation of growth. Similar observations on the effect of carbon dioxide on the wilting of plants have been made by other investigators. CHANG and LOOMIS (5) found that carbon dioxide reduced the absorption of water and nutrients by wheat, maize, and rice when compared with aerated cultures of the same plants.

Concentrations of carbon dioxide between zero and 15 per cent. produced no appreciable influence on cotton tap-root elongation. Concentrations of 15 per cent. carbon dioxide occur very rarely in the soil air, as determined by gas analysis. In a previous paper (10) it was pointed out that concentrations of carbon dioxide may be very high adjacent to the surfaces of roots under some conditions, although the occurrence of such concentrations might not be revealed by a gross analysis of the soil air. It is possible that concentrations of even 60 per cent. carbon dioxide are sometimes attained. The concentrations of carbon dioxide at root surfaces under various soil conditions must be determined before the influence of carbon dioxide on root growth in a soil can be evaluated.

Concentrations of carbon dioxide from zero to 100 per cent. were studied in order to determine the influence of all possible concentrations which might possibly influence root growth in the soil. Normal root growth occurred in the absence of carbon dioxide. It does not appear possible, therefore, that minimum concentrations of carbon dioxide can be too low to seriously influence seedling cotton root growth. Further work should be done on the minimum requirements of plant roots for carbon dioxide.

Carbon dioxide is necessary for the growth of many microorganisms. JAHN (9) observed that the growth of the protozoan, Chilomonas, was reduced to less than one-fifth in carbon dioxide-free air when compared to ordinary air. LONGSWORTH and MACINNES (11) found that carbon dioxide was necessary for the growth of *Lactobacillus acidophilus*. GLADSTONE, FILDES, and RICHARDSON (8) observed that the passage of carbon-dioxide-free air through cultures of various bacteria stopped growth in some cultures, but not in others. In cultures not inhibited, carbon dioxide was probably formed within the cells themselves. Perhaps the reason cotton root growth was not influenced by the absence of carbon dioxide in the gas mixtures was because all cells of the roots were in contact with adjacent respiring cells; thus no cells were completely subjected to an atmosphere containing zero carbon dioxide, regardless of the composition of the gas mixture around the roots.

Roots in the same environment did not elongate at the same rate from day to day. CANNON and FREE (4) likewise reported similar variations in what appeared to be identical plants. As far as could be ascertained, there was no relationship between root growth, leaf initiation, or leaf area.

Summary

A study was made of the oxygen and carbon dioxide requirements of cotton seedling roots in nutrient solutions.

Optimum oxygen concentrations for seedling root elongation of cotton appeared to lie between seven and one-half and 21 per cent. oxygen when carbon dioxide was constant at 10 per cent. The greatest rate of root elongation for any 24-hour period was 67 mm. in 21 per cent. oxygen and 10 per cent. carbon dioxide with culture temperature at 30° C.

The minimum oxygen requirement for cotton root elongation at 28° C. appeared to lie between one-half and one per cent. oxygen. Absence of oxygen around roots did not appear to be very harmful under the conditions studied. When air containing 21 per cent. oxygen replaced nitrogen gas (zero per cent. oxygen), new branch roots were initiated, and some of the old branch roots commenced to grow again.

Ninety or 100 per cent. oxygen resulted in reduced root elongation. Roots produced under such conditions were readily parasitized by chytrids and species of Fusaria.

Tap-root elongation at each concentration of oxygen was similar whether nitrate or ammonium nitrogen was used in the culture solution. Vegetative growth appeared improved with the nitrate nitrogen. More nitrate nitrogen than ammonium nitrogen was taken up by the plants.

The optimum concentration of carbon dioxide appeared to range between zero and 15 per cent. when the oxygen concentration was maintained at 21 per cent. The absence of carbon dioxide did not appear to affect root elongation. Concentrations of 60 per cent. carbon dioxide and above prevented all root growth. Root growth was reduced to 30 and 45 per cent. carbon dioxide, and the roots were rather thick.

Shoot growth appeared to be uniform in both height and fresh weight from zero to 30 per cent. carbon dioxide, reduced at 45 per cent. carbon dioxide, and greatly reduced at 60 per cent. carbon dioxide and above. The shoots frequently wilted at 60 per cent. carbon dioxide and above, especially when the plants were in direct sunlight and the temperature was over 30° C.

The writers conclude that the cotton plant is able to withstand anaerobic soil conditions and that this ability is, perhaps, related to the translocation of oxygen from the tops to the roots.

Mississippi Agricultural Experiment Station
State College, Mississippi

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ABSORPTION OF WATER THROUGH SUBERIZED ROOTS OF TREES

PAUL J. KRAMER

(WITH ONE FIGURE)

It is generally assumed that absorption of water and solutes by plant roots occurs principally in the unsuberized region back of the root cap where root hairs are usually produced. It seems probable, however, that under some conditions considerable absorption must occur through the suberized regions of roots, especially those of woody plants. For example, root elongation of most trees and shrubs almost or entirely ceases in cold or dry soil, and few or no unsuberized root tips exist at such times. Nevertheless many evergreen species such as pine, privet, holly and citrus lose considerable water by transpiration during such periods. The water lost during periods of little or no root elongation presumably is replaced by absorption through the suberized surfaces of fully differentiated, mature roots. Several investigators have presented indirect evidence that this actually occurs. CRIDER (**5**) reported absorption of nitrate and phosphate through roots of citrus and vitis after all root tips had been removed and the cut ends sealed. CHAPMAN and PARKER (**2**) observed absorption of nitrate by orange roots which had turned brown and were at least partially suberized. COLBY (**4**) stated that apparently apple trees can absorb enough water through suberized roots to prevent wilting but not enough for shoot growth. NIGHTINGALE (**7**) found that potted apple and peach trees grown at soil temperatures of 45 and 95° F. produced no new roots and some of the old roots even died at 95° F. Nevertheless these trees absorbed water as indicated by the fact that they leafed out and made some shoot growth. Whatever absorption occurred during these experiments must have been through the old suberized roots, or even, in some instances, through dead roots.

In spite of the strong presumptive evidence little direct evidence of water intake through suberized roots has been obtained. In fact the only direct measurements known to this writer are those made by HAYWARD, BLAIR, and SKALING (**6**) under laboratory conditions on roots of sour orange seedlings. It was decided therefore to make a series of measurements of absorption through suberized roots of trees, especially pines, growing in the open.

Methods and results

Potometers were made from both glass and rubber tubing. They are shown in figure 1. The glass potometers were slipped over the ends of cut roots and sealed over split stoppers bored to fit snugly around the roots, thus surrounding the root with a water chamber about 2.5 cm. long and 1.0 cm. in diameter. The joints were made watertight with grafting wax or Fisher's Pyseal, the latter being particularly useful in hot weather. Each potometer had two side tubes. To one was attached a small pipette graduated in 0.05

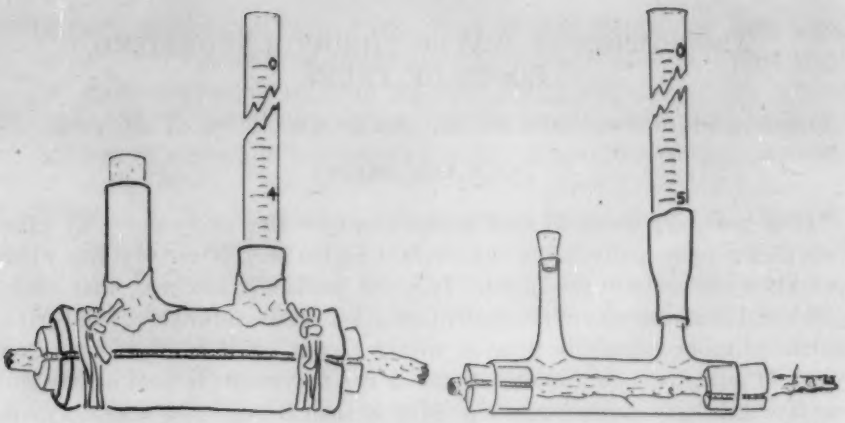

FIG. 1. Drawings of root potometers. Rubber potometer on left, glass potometer on right.

ml. divisions while the other side tube permitted the escape of air during the filling of the potometer, after which it was closed with a stopper. The rubber potometers were made from thick-walled rubber tubing and side tubes were provided by sealing pieces of smaller bore rubber tubing into holes cut near each end. The potometers were split lengthwise so they could be slipped over attached roots without cutting them or uncovering more than 20 or 30 cm. The water chamber was formed by first fastening two split, one-hole stoppers around the roots, then sealing the ends of the potometer to them and tying them tightly with heavy rubber bands. Rubber to rubber joints were sealed with rubber cement and then coated with grafting wax or Pyseal. The diameter of the potometers varied according to the diameter of the roots on which they were placed. Tests made in the laboratory indi-

TABLE I

ABSORPTION OF WATER BY ATTACHED ROOTS OF SHORTLEAF PINE IN CU. MM. PER SQ. CM. PER HOUR

	TREE NO.	POTOMETER NO.	DIAMETER OF ROOT	DISTANCE FROM TRUNK	AVERAGE DAY RATE	NO. OBS.	AVERAGE NIGHT RATE	NO. OBS.	DAY/NIGHT
			mm.	*cm.*	*mm.*³		*mm.*³		
June	1	1	4.8	30	8.35	8	3.52	7	2.3
	2	1	7.0	155	0.99	3	0.55	4	1.8
		2	8.0	248	3.37	8	0.90	7	3.7
	3	1	17.0	36	2.57	6	0.50	7	5.1
		2	4.7	210	1.57	3	1.13	4	1.3
				Average	3.37	....	1.32	....	2.8
August	1	1	5.0	25	1.86	2	0.78	2	2.4
	2	1	3.2	20	2.07	2	1.45	2	1.4
	3	1	3.2	25	1.28	2	0.53	2	2.4
				Average	1.73	....	0.92	....	2.1

cated that the volume changes caused by change in temperature were not large enough to interfere with measuring absorption.

The roots used varied from 3.0 to 17.0 mm. in diameter and belonged to shortleaf pine (*Pinus echinata* Mill.) trees 8 to 15 feet in height. Care was taken to avoid injury to the surface of the roots, and potometers were located on segments free of side branches and visible injuries. Results of several days observations are summarized in table I. Measurable volumes of water were absorbed during both day and night by roots of all diameters. Simultaneous observations were made with potometers attached at various distances from the trunk, but no correlation was apparent between absorption and distance of potometer from the tree. When measurements were made in June the soil was very dry, the temperature was high, and conditions were favorable for high transpiration; but the August measurements were made a few days after the soil had been thoroughly wetted by rains, and atmospheric conditions were less favorable for transpiration. Both series were made on the same trees. As would be expected, more water was absorbed from the potometers per unit of root surface when the soil was dry than when

TABLE II

AVERAGE DAY RATE OF ABSORPTION OF SHORTLEAF PINE ROOTS IN CU. MM. PER SQ. CM. PER HOUR

From water	3.37	Average of 28 determinations
From soil	2.63	Average of 6 determinations

it was moist. The results are summarized in table I. Measurement of water intake at two-hour intervals during the day indicates that the rate of absorption is probably closely related to the rate of transpiration, but lags behind it somewhat so that considerable absorption occurs at night.

Attempts were also made to measure the rate of absorption from moist soil. A layer of soil moistened to field capacity was held in close contact with roots by means of pieces of rubber tubing which enclosed soil and root. The rate of absorption from soil initially wetted to field capacity averaged about as high as absorption from water (table II). These experiments lasted 12 hours, from 6 A.M. to 6 P.M. Presumably the rate would decrease markedly after the available water was removed from the soil in immediate contact with the roots.

A comparison of the rates of water movement through pine and hardwood roots was also made. It was decided that this could not be done on roots attached to trees because it would be impossible to determine whether differences in rate of water intake resulted from differences in permeability or from differences in rate of absorption of the trees to which the roots were attached. Comparisons were therefore made in the laboratory by sealing one end of root segments and attaching the other end to a vacuum line and immersing the root segments in water. Thus the permeability to water could be compared under similar conditions. The species used were dogwood and

shortleaf pine. The data obtained are summarized in table III and indicate that dogwood roots are considerably more permeable to water than pine roots.

TABLE III

RELATIVE PERMEABILITY OF PINE AND DOGWOOD ROOTS. ROOT SEGMENTS WERE ATTACHED TO A VACUUM LINE AND SUBJECTED TO 30 CM. PRESSURE. RATES IN CU. MM. PER SQ. CM. PER HOUR

SPECIES	NUMBER OF DETERMINATIONS	AVERAGE RATE
		mm.³
Shortleaf pine	19	9.0
Dogwood	5	15.6
Yellow poplar	4	101.4

Discussion

These results indicate that appreciable quantities of water can be absorbed through suberized roots, even through roots one or two cm. in diameter which are covered with a relatively thick layer of bark. No information is given, however, as to the manner in which water passes through the bark. HAYWARD, BLAIR, and SKALING (6) stated that water entered orange roots chiefly through the numerous lenticels. Studies of the path of water movement in pine, yellow poplar and sweet gum have been made by a colleague and will be reported in another paper (1).

It is not intended to depreciate in any way the importance of unsuberized root tips and of root elongation in the absorption of water. There are times, however, when the soil is too cold or dry for root elongation to occur, and few or no unsuberized root tips exist. At such times absorption through suberized roots may be of major importance. The number of small suberized roots in the soil under a forest stand is large enough to possess considerable surface and absorb an appreciable amount of water (3). Since the soil is usually moist during the winter when root growth is slowest, conditions are such that most or all of the water required by evergreen trees probably can be absorbed through the mature, suberized roots. Even in the summer some water is doubtless absorbed through them, particularly when a rain follows a drought during which root elongation has ceased, and the roots have become suberized to their tips. Several days would be required following a rain for root growth to be resumed, but absorption through the older portions of the roots doubtless begins immediately.

Absorption through older roots of herbaceous species doubtless also occurs under certain conditions. In a recent study (9), for example, it was found that the seminal roots of a number of perennial grasses remained alive for 3.5 to 4 months. Although the cortex had sloughed off the older portions of these roots, it seems probable that even the decorticated portions function as absorbing surfaces, at least in moist soil. It has been reported (8) that certain grasses have perennial roots; that is, most of the roots continue to

function the second season. Considerable absorption must occur through such roots. It seems probable, therefore, that absorption through older roots in which secondary growth has occurred is a fairly common occurrence, especially at times when few roots are elongating.

Summary

The rate of water absorption through suberized roots of shortleaf pine was determined by attaching potometers to roots of small trees growing in the open. The roots used varied in diameter from 3.2 to 17.0 mm. and were covered with thick layers of cork. Measurable absorption occurred through all roots studied. Absorption of water from moist soil by suberized roots also occurred.

Comparison of the rates of water movement through segments of pine and dogwood roots indicate that dogwood roots are much more permeable to water than pine roots.

Absorption of water through suberized roots is probably of vital importance in evergreen trees during the winter when root growth is much reduced or ceases and the roots become suberized to their tips. It is probably also of some importance in the summer, particularly immediately following a drought which has stopped root elongation.

DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

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MINERAL REQUIREMENTS OF *LEMNA MINOR*

ROBERT A. STEINBERG

Introduction

The use of *Lemna minor* Link as a test organism for the micronutrient requirements of the higher plants was undertaken by the writer as a check on similar data with the fungus *Aspergillus niger* v. Tiegh. It was possible to demonstrate that the macro- and micronutrient needs of both were similar, except that Lemna also required calcium and boron (**1**). The mineral and acidity requirements of Lemna were identical with those of other higher green plants.

In Lemna cultures with *fixed, minimal quantities of salts,* the ratio of yield to total macronutrient salts was 0.9, a value termed "efficiency of salt utilization" by the writer. This corresponded to a total of 1,113 mg. of macronutrients per gram of yield, each salt of the nutrient solution having been adjusted to be at the experimental minimum for maximum yield. Addition of 0.5 per cent. sucrose, nevertheless, increased the ratio to 2.3 though the quantity of salts remained unaltered. The macronutrient salt requirement was thereby decreased to 434 mg. per gram of yield. Aspergillus, however, reaches an efficiency of 9.6, which is equivalent to 106 mg. salts per gram. The lower efficiency of Lemna may be intrinsic but might be only a reflection of secondary requirements, such as buffering of the nutrient solution and insufficient energy (low photosynthesis) for efficient absorption.

An effort has been made to obtain an improvement in the results previously reported. Numerical data on the effects of copper are also included.

Methods

Lemna minor Link was grown on 50 ml. of nutrient solution in 200-ml. Erlenmeyer flasks under aseptic conditions. Illumination was continuous at 500 foot-candles supplied by 3,500° C. white fluorescent lamps. The temperature was 25° ± C. Photosynthesis is the limiting factor for growth under these conditions (**1**). Only quartzware was used. Nutrient solutions were prepared with water twice redistilled in quartz and standard reagent chemicals having a purity sufficient for chemical analyses. Micronutrients, however, were almost spectroscopically pure chlorides. The sucrose contained 0.0011 per cent. ash. Each experiment was started with a single plant (having two fronds) per flask. The roots usually varied from 2 to 5 mm. in length. Duration of growth was 13, 14, or 27 days with continuous illumination. No change was made in the solutions of the individual flasks for the duration of the experiments. The plants were dried at 103–5° C. for 3 to 4 hours before weighing.

Results

Effects of micronutrient deficiencies

The effects of micronutrient deficiencies on yields are shown in table I. Selected reagent chemicals were used in various combinations. The solutions used were adjusted to minimum salt content for maximum yield. Sharp diminutions in growth were readily obtained on omission of iron, manganese, molybdenum, and boron. Results with zinc, copper, and gallium were less clear cut.

TABLE I

Effects of micronutrient deficiencies on growth of Lemna with selected reagent chemicals

Element omitted	Selected reagent chemicals*											
	13 days pH = 4.17			13 days pH = 4.06			13 days pH = 4.10			27 days pH = 4.30		
	Yield	Relative yield	Appearance†	Yield	Relative yield	Appearance†	Yield	Relative yield	Appearance†	Yield	Relative yield	Appearance†
	mg.	%		*mg.*	%		*mg.*	%		*mg.*	%	
None	13.2	100.0	4,M	15.6	100.0	4,M	14.0	100.0	4,M	60.1	100.0	4,M
Fe	2.5	18.9	1,S	3.3	21.2	2,S	2.1	15.0	1,T	3.9	6.5	1,S
Zn	12.1	91.7	4,M	13.3	85.3	4,M	11.7	83.6	4,M	44.7	74.4	2,M
Cu	10.6	80.3	4,M	13.9	89.1	4,M	7.8	55.7	4,M	34.8	57.9	4,M
Mn	3.1	23.5	2,T	2.9	18.6	2,S	1.7	12.1	1,T	2.5	4.2	1,T
Mo	4.3	32.6	2,M	3.2	20.5	2,S	3.1	22.1	2,S	40.5	67.4	4,M
Ga	12.2	92.4	4,M	15.4	98.7	4,M	8.9	63.7	4,M	55.7	92.7	3,M
B	5.4	40.9	5,T	9.2	59.0	5,S	5.2	37.1	5,S	43.4	72.2	5,S

* First 3 experiments: Water, 1 liter; KNO_3, 50; KH_2PO_4, 50; $MgSO_4 \cdot 7H_2O$, 30; Fe, 0.20; Zn, 0.05; Cu, 0.01; Mn, 0.05; Mo, 0.02; Ga, 0.02; B, 0.02; Ca, 4.0 mg. Last experiment: Water, 1 liter; KNO_3, 300; KCl, 50; KH_2PO_4, 70; $MgSO_4 \cdot 7H_2O$, 45; Fe, 0.40; Zn, 0.10; Cu, 0.02; Mn, 0.20; Mo, 0.06; Ga, 0.04; B. 0.06; Ca, 8.0 mg.

† Color is rated from 0 to 5 (normal green); and size of fronds designated as L = large, M = medium, S = small, T = tiny.

Symptoms of micronutrient deficiency followed a parallel course. Normal plants had large green fronds with flat, rounded apices and long, light green roots. With insufficient iron, the plants remained adherent; the fronds were tiny with distorted apices and displayed a diffuse chlorosis, usually progressing from apex to base of frond. Roots were short, light green, and sometimes distorted. Deficiencies in zinc, gallium, and particularly copper were sporadically accompanied by a general diffuse chlorosis of fronds very similar to that shown in absence of iron.

In the absence of manganese the plants remained adherent and had very small fronds with sharply defined white margins and elongated blotches giving a green "trident effect" on all but the base, and occasional red spots. Roots were short, twisted, and dark green. Omission of molybdenum also resulted in adherent plants with small fronds, many of which had acute up-

turned apices. Chlorosis was diffuse with white blotches merging into green and often accompanied by narrow marginal streaks of dark green. The roots were long, twisted, and very dark green. Non-addition of boron led to plants markedly adherent, having tiny and very dark green fronds with occasional acute apices and red spots. Roots were very short, dark green, and kinky.

Nutrient solution purification

Table II contains experiments illustrating the effects of nutrient solution purification (1) on micronutrient deficiencies. Improved results were obtained with iron, zinc, and copper as compared to unpurified solutions composed of selected reagents. Results with gallium were slightly better when calcium phosphate was used instead of calcium carbonate. Details of this process will be found in the publication cited above. The removal to a greater or lesser extent of residual traces of iron, zinc, copper, etc., impurities from the reagent chemicals is accomplished by neutralizing the complete nutrient solution with an excess of $CaCO_3$, $CaHPO_4$, CaO, or the corresponding magnesium salts, in the presence of excess phosphate. The sediment formed is an important factor in the treatment, since it serves also as an adsorbent for micronutrient impurities. The clear solution is used and the sludge rejected.

These solutions contained: water, 1 liter; KNO_3, 400; K_2HPO_4, 440; $MgSO_4 \cdot 7H_2O$, 350; $CaCO_3$, 250; and $CaHPO_4 \cdot 2H_2O$, 750 mg. After heating in a steamer for 20 minutes, the solutions were filtered cold, and 2.0–2.5 ml. of N/1 hydrochloric acid added. Micronutrients per liter then added were: Fe, 0.40; Zn, 0.04; Cu, 0.01; Mn, 0.075; Mo, 0.02; Ga, 0.02; B, 0.04 mg.

Efficiency of salt utilization

Previous work (1) had indicated that addition of sucrose to a Lemna culture resulted in an increase in growth even though each nutrient component was presumably at the experimental minimum for maximum normal growth. The optimum macronutrient mixture of 32.5 mg. salts that was essential for a yield of 29.2 mg. without sucrose sufficed for the production of 74.9 mg. on addition of 0.5 per cent. sucrose. This is equivalent to a decrease in salt requirements for growth with an increase in level of carbon nutrition and is, therefore, referred to as an increase in "efficiency of salt utilization." The corresponding ratios 29.2/32.5, or 0.9; and 74.9/32.5, or 2.3 are reciprocals of salts per unit of yield.

Salt utilization ratios with Lemna have been given further attention in table III. The data summarized should suffice to demonstrate each of several points. Normal growth is dependent on quantity ratios of nutrients irrespective to total quantity. Available sugar is closely correlated with total quantity of salts required for growth. Increased salt utilization efficiency with increased carbon nutrition takes place with chlorotic as well as normal plants.

An intensity of 500 foot-candles is known to be sub-optimal for growth

TABLE II

EFFECT OF NUTRIENT SOLUTION PURIFICATION ON RESPONSES OF LEMNA TO MICRONUTRIENT DEFICIENCIES (14 DAYS)

Element omitted	$CaCO_3$ purification						$CaHPO_4 \cdot 2H_2O$ purification								
	Sucrose 0.1% pH = 4.01			No sucrose pH = 4.05			Sucrose 0.1% pH = 4.32			No sucrose pH = 3.97			Filtered through 5 cm. $CaHPO_4 \cdot 2H_2O$, no sucrose pH = 4.01		
	Yield	Relative yield	Appearance*	Yield	Relative yield	Appearance*	Yield	Relative yield	Appearance*	Yield	Relative yield	Appearance*	Yield	Relative yield	Appearance*
	mg.	%		*mg.*	%		*mg.*	%		*mg.*	%		*mg.*	%	
None	44.7	100.0	4, M	15.8	100.0	4, L	29.7	100.0	4, L	22.0	100.0	4, L	16.1	100.0	4, L
Fe	1.8	4.0	1, S	0.6	3.8	1, T	1.5	5.1	1, S	1.2	5.5	2, S	1.2	7.4	2, S
Zn	21.2	47.4	4, L	7.6	48.1	4, M	22.1	74.4	4, L	11.4	51.8	4, L	10.2	63.0	4, L
Cu	16.0	35.8	4, L	10.6	67.1	4, M	24.3	81.8	3, L	12.4	56.4	4, L	12.6	77.8	4, L
Mn	3.3	7.4	2, S	1.0	6.3	1, T	5.3	17.8	2, S	1.3	5.9	2, T	0.8	4.9	1, S
Mo	27.2	60.9	4, L	6.1	38.6	4, M	10.1	34.0	4, L	7.5	34.1	4, T	14.7	90.7	4, L
Ga	42.1	94.2	3, L	15.2	96.2	3, M	25.9	87.2	4, L	16.6	75.5	4, L	13.1	80.9	4, L
B	16.5	36.9	5, S	12.1	76.6	4, M	27.2	91.6	4, L	12.0	54.5	5, S	12.9	79.6	4, L

* See footnote, table I.

of Lemna (1) which requires 1500 foot-candles. Air enriched with CO_2 is ineffective in increasing yields except in experiments of over 2 weeks duration when the leaf surface increases to the point where air diffusion through the cotton plugs of the flasks becomes inadequate. Light is the limiting factor for growth during the early stages of the experiment and carbon dioxide during the later stages. Sucrose was effective at all stages in increasing growth, whereas manipulation of macro- and micronutrients was ineffective, as were also addition of trace elements or vitamins. The action of

TABLE III

EFFICIENCIES OF SALT UTILIZATION (YIELD/TOTAL AVAILABLE MACRONUTRIENT SALTS) OF *Lemna minor* GROWN FOR 27 DAYS AT 25° C. WITH 500 FOOT-CANDLES OF FLUORESCENT LIGHT

NUMBER	MILLIGRAMS PER CULTURE OF 50 ML.				MILLIGRAMS YIELD PER CULTURE	SUPPLEMENT	YIELD DIVIDED BY TOTAL AVAILABLE SALTS
	KNO_3	KH_2PO_4	$MgSO_4 \cdot 7H_2O$	TOTAL SALT			
	mg.	*mg.*	*mg.*	*mg.*	*mg.*		
1*	15.0†	3.5	2.25	23.25	61.0	.....	2.6
2	17.5	10.0	5.0	32.5	63.2	.....	1.9
3	7.5‡	3.25	2.0	15.75	47.6	.....	3.0
4	7.5‡	3.25	2.0	15.75	191.0	0.5% sucrose	12.1
5	4.5	3.0	2.0	9.5	47.8	.....	5.0
6	4.5	3.0	2.0	9.5	60.8	CO_2	7.2
7	4.5	3.0	2.0	9.5	85.6	*A. niger* vapors	9.0
8	4.5	3.0	2.0	9.5	106.4	*A. niger* vapors	11.2
9	6.0‡	3.25	2.0	14.25	40.6	.....	2.9
10	6.0‡	3.25	2.0	14.25	83.5	0.1% sucrose	5.9
11	6.0‡	3.25	2.0	14.25	134.1	0.2% sucrose	9.4
12	6.0‡	3.25	2.0	14.25	173.6	0.3% sucrose	12.2
13	6.0‡	3.25	2.0	14.25	198.6	0.4% sucrose	13.9
14	6.0‡	3.25	2.0	14.25	222.7	0.5% sucrose	15.6
15	6.0‡	3.25	2.0	14.25	232.5	0.6% sucrose	16.3

* Experimentally determined to contain a minimal quantity of each salt for maximum normal growth under the specific environmental conditions employed. All other cultures were chlorotic. Micronutrient concentrations as in table I, last experiment.

† Plus 2.5 mg. KCl or KNO_3 per culture.

‡ Plus 3.0 mg. $Ca(NO_3)_2 \cdot 4H_2O$ per culture.

sucrose is, therefore, assumed to depend on an increase in the level of carbon nutrition of Lemna.

The minimal quantities of macronutrient salts required for maximum normal growth are shown in experiment 1. The yield was 61 mg. per culture and required 23.25 mg. of macronutrients; i.e., a ratio of 2.6. Neither an increase (#2) nor a decrease (#3) in individual or total components of the solution increased yield but did cause chlorosis. Addition of sucrose (#4) brought about a 3-fold increase in yield and a 4-fold increase in salt utilization ratio without eliminating chlorosis. Experiments 5 to 8 illustrate similar increases in salt utilization ratios with CO_2 and with vapors from cultures of *Aspergillus niger* v. Tiegh. The remaining experiments show the effect of increasing quantities of sucrose on yield and utilization ratios

despite the fact that nutrition was abnormal and the plants chlorotic. Sucrose caused a 6-fold increase in yield despite chlorosis.

These data would indicate, therefore, that nutrient salts required for growth of Lemna are, for reasons unknown, far in excess of the plant's actual requirements for formation of tissue. Usually 38.5 per cent. of salts (1/2.6) must be provided on the basis of anticipated yield. With sucrose, 6.1 per cent. of salts (1/16.3) sufficed. This value approaches that mentioned by PFEFFER in his treatise of plant physiology, where he points out that a minimal salt solution for the oat plant should require only about 2.5 per cent. salts per unit of yield. This computation was based on the experimental optimum for each element.

Though it has been possible to approach this value with Lemna, the problem remains of doing so without causing chlorosis. The causes of these chloroses were sought in experiments with macronutrient salts and trace elements, but unsuccessfully. The further addition of KNO_3 [also $Ca(NO_3)_2$ or KCl] prevented chlorosis under these conditions but only in association with marked decreases in efficiency of salt utilization.

Discussion

Improved results with micronutrient deficiencies were obtained through selection of nutrient salts and their use in minimum quantities. Decreases in growth due to their omission from the nutrient solution were accompanied as a rule by specific symptoms in the case of iron, manganese, molybdenum, and boron. Symptoms of zinc, copper, and gallium deficiency were sporadic and consisted in uniform chloroses of older fronds.

The salt utilization studies with Lemna have been based on the use of limited quantities. That is to say, the quantities initially supplied were used at the experimental minima for maximum normal growth and were not replenished to compensate for depletion. Quantity ratios and total quantity were found to be important factors for growth under these conditions (**2**). An indefinite and variable proportion of the total salts supplied did not appear to function primarily in the elaboration of tissue by the plant. The apparent increase in effectiveness of the salt supply in maintaining growth on addition of sugar would indicate this to be so and also that maximum efficiency of salt utilization would be reached at the optimum carbon nutrition level of the plant. Hindrance of salt absorption because of low carbon nutrition level and buffer requirements may be factors in higher mineral needs. The carbon nutrition level, therefore, appears to be the determining factor for total salt requirements in the case of Lemna as well as Aspergillus (**2**).

Summary

Lemna minor Link was grown in aseptic culture for 13, 14, or 27 days at 25° C. with continuous light (500 foot-candles, fluorescent lamps) using fixed, limited quantities of nutrients. The effects of deficiencies in micronutrients are described. Efficiency in salt utilization was found to be depen-

dent on the carbon nutrition level of the plant and reached a maximum value of 16.3 (yield/salt) or the equivalent of 6.1 per cent. salts for each unit of yield. Quantity and quantity ratios of nutrient salts were important factors in growth.

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

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INFLUENCE OF EXTERNAL AND INTERNAL FACTORS ON GROWTH HORMONE IN GREEN PLANTS[1]

FELIX G. GUSTAFSON

Over a period of several years the writer has made numerous determinations of growth hormone content in plants, as influenced by external and internal factors. The external factors studied were mineral nutrition, light, temperature, and parasites; internal factors studied were chlorophyll, dwarfness, age of the plant, and position on the plant of an organ. Other studies of this sort have been published and will be referred to under the appropriate section.

Materials and methods

Hormone tests were made by the Avena method (**17**) on extracts from the plant. Several methods have been employed in making the extracts, but differences in extraction method are not important because comparisons are made only within an experiment or between experiments conducted on the same day in an identical manner, a procedure obviating the necessity of making calculations in terms of indoleacetic acid equivalent or any other equivalent. In most of these experiments direct comparisons are made of Avena curvatures obtained with different materials when the procedure of extraction and auxin determination were identical and carried out at the same time with the same lot of Avena plants. In most of these experiments the same amount of plant material was used, and in the final analysis the same dilution was used; i.e., the same amount of agar was added to comparable amounts of extract. This procedure eliminates many objections that have been raised against some of the methods used in stating auxin content in plants.

Several different plant species have been used, and these will be mentioned as they come up; the main experimentation has been made with the John Baer tomato, though corn has also played an important part in the investigation.

Results

INFLUENCE OF SOIL FERTILITY ON AUXIN CONTENT OF TOMATO PLANTS

In a study of growth hormones, a comparison between growth and hormone content is very important; as one means of regulating growth is by varying the mineral nutrition, this method has been used. AVERY, BURKHOLDER, and CREIGHTON (**1, 2**) found that vigorously growing sunflower and Nicotiana plants had a high concentration of auxin whereas poorly growing plants had less hormone; and that field-grown Nicotiana plants showing

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deficiency symptoms had as much hormone as controls. SKOOG (12), on the other hand, found that zinc-deficient tomato plants had much less hormone than plants supplied with zinc. WENT (18) found a slight reduction of hormone with low nutrition of tomato.

In the present experiments, John Baer tomatoes were grown in soil under conditions similar to those used in commercial greenhouses except that the plants were grown in large pots instead of in the ground. Three soils were used: soil I, composed of ordinary field soil without any addition of fertilizers; soil II, an artificial compost made from turf, manure, dried blood, German peat, and $(NH_4)_2SO_4$, and permitted to decompose for a period of three years; and soil III, which was soil II to which had been added one 3½-inch pot of bone meal and one of Vigoro per bushel of soil. During the early part of the experiment the plants were grown in 6-inch pots but as they became larger they were transferred to 9-inch pots. At the time of repotting, soils II and III were refertilized.

TABLE I

INFLUENCE OF SOIL FERTILITY ON THE AUXIN CONCENTRATION IN TOMATO PLANTS. DEGREE OF CURVATURE OF THE AVENA COLEOPTILES DENOTES HORMONE CONTENT. FOR FURTHER INFORMATION ON THE METHOD OF INDICATING HORMONE CONCENTRATION SEE THE TEXT

EXP.	DATE	CURVATURE OF AVENA COLEOPTILES		
		SOIL I	SOIL II	SOIL III
211	11/23/42	13.8	14.9	15.1
212	12/ 2/42	4.8	6.8	8.0
220	1/11/43	3.9	9.4	6.9
227	3/31/43	1.6	4.9	5.1

Four determinations of auxin were made; for each determination two or three leaves from near the tip of the plants were used. On November 23, when the first sampling was made, the plants in soil I were about 4 feet tall, thin, and spindling with the lower leaves yellow and with one or two fruits per plant. The plants in soils II and III were more vigorous with dark green leaves but had no fruits. On December 2, when a second collection of material was made, the plants were essentially in the same condition as in November. After gathering the material necessary for the test, the plants were pruned and repotted in 9-inch pots. The third collection was made on January 11, and at this time the plants in soil I showed signs of extreme starvation; they had no flowers or flower buds, the leaves were small, and the veins were purple (lack of phosphorus); the other plants had flowers, flower buds, and fruits, and the leaves were large and green. The last collection was made on March 31; at that time the plants in soil I had very small leaves, veins and stems showed deep purple color, and the stems were thin and not very tall. The other plants were vigorous, with many flowers and large green leaves, the plants in soil III being the most vigorous.

The relative hormone content is denoted by the degree of curvature of

Avena coleoptiles. In a particular experiment the amount of plant material used was the same from each soil, and the amount of agar added to the extract was the same, so a direct comparison can be made between the plant lots.

It is evident that poor soil produces poor plants and that poor plants have a much lower hormone content than vigorous plants. It is equally evident that increasing the soil fertility beyond a certain point does not increase the vigor of the plants or their hormone content. In the early part of the experiment the plants grown in soil I, though smaller than the others, were still quite vigorous, and at that time their hormone content was very near to that of the others; but as the nutrient of the soil became depleted, the hormone content decreased greatly. At the beginning of the experiment, soils II and III were too rich for the setting of fruits; but later, as the soils became depleted, the plants fruited abundantly. The repotting of the plants, and especially the addition of fertilizers to soils II and III at that time, increased the hormone content of the plants slightly. The experiments further bring out the fact that phosphorus starvation is associated with low hormone content; but as the plants in soil I were lacking in vigor due to lack of other elements also, not too much emphasis should be placed on the lack of phosphorus.

Influence of light upon the auxin content of green plants

This experimentation was initiated to discover whether in hormone studies time of day at which plant material is collected has any significance; but later the experiments were more concerned with the influence of light upon the hormone concentration.

The preliminary experiments in which material was collected in the morning and late afternoon, from plants grown either in the field or in the greenhouse, were not very satisfactory. In a general way it can be stated that, when there was any difference between the two collections, the afternoon collection had a lower hormone content than the morning collection if the light intensity was great, as it would be on a sunny day in the summer; but if, on the other hand, the day was cloudy, or if the time was winter, the evening collection was likely to have more hormone. In all of these experiments, tomato plants about 3 to 4 feet tall were used, grown either in the field or greenhouse. While not very satisfactory, these experiments nevertheless showed the desirability of collecting the material at the same time each day and preferably around noon. This schedule has been followed quite consistently in this laboratory for some years.

The experiments just mentioned led to others in which plants of different sizes grown in the greenhouse were kept in the dark for various lengths of time. Some investigators (**3**) had shown that in Nicotiana there was a rapid disappearance of the hormone when the plants were kept in the dark room, while others (**5**) had shown that seedlings grown in the dark and then exposed to light suffered a loss of hormone as a result of the light stimulation

and still others (**18**) have shown that plants in dark have more hormone than plants in the light. These results seem, at least on the surface, contradictory.

A group of similar plants were selected and half of them placed in a darkroom while the others were left in the greenhouse (table II). In the darkroom, which was connected to the greenhouse by a ventilator, a fan was continuously sucking in air which left the room by another opening. Thus, except for the middle of hot days, the temperature and relative humidity were the same in the two rooms. As will be noticed, the time of these experiments extended over a period of one year, a period during which various light and temperature conditions were encountered. Except for three experiments, the John Baer tomato was used as the experimental plant.

TABLE II

GROWTH HORMONE CONTENT IN PLANTS SUBJECTED TO NORMAL LIGHT CONDITIONS AND TO CONTINUOUS DARKNESS FOR DIFFERENT LENGTHS OF TIME

EXP.	DATE	CURVATURE OF AVENA PRODUCED BY PLANTS FROM		HOURS IN DARK	KIND AND SIZE OF PLANTS
		GREEN-HOUSE	DARK-ROOM		
263	1/24/44	6.3	7.1	26	Tomato, 2 ft. tall
			7.3	50	
264	1/31/44	11.0	8.4	21	Tomato, 3–4 feet
			6.7	45	
280	5/15/44	10.2	13.2	20	Tomato, 3 feet
			9.9	49	
305	11/13/44	15.8	18.6	50	Corn, 17 days old
309	11/27/44	4.6	6.6	49	Tomato, 6–8 in. tall
311	12/ 4/44	4.0	4.2	65	Tomato, 8–10 in. tall
312	12/11/44	1.0	17.6	72	Corn, 14 days old
313	12/11/44	0.9	13.0	48	Tomato, 8–10 in. tall
317	1/ 3/45	7.8	14.2	65	Corn, 6–7 in. tall

In each experiment the same amount of plant material was used from both lots of plants and the same amount of agar was added to the extracts, so that the curvatures produced in the Avena plants are strictly comparable.

In all but one experiment given in table II, the plants in the dark have a higher hormone content than the plants in the light. Sometimes the difference is very slight, at other times very great. This is very different from the findings of AVERY, BURKHOLDER, and CREIGHTON (**3**), who noted that there was a depletion of the hormone in Nicotiana plants kept in the dark for some days. On the other hand, BURKHOLDER and JOHNSTON (**5**) found that plants removed from darkness to light lost hormone. The interpretation of all of these different results might be that hormone is destroyed by light, that in intense light more hormone is destroyed than is produced, and that in the dark the hormone is conserved. If the plant has an abundance of precursor, as has been found for tomato (**6**) and corn (**4**), there would be an increase in the hormone content in the plants in the dark (table II); if

the plant does not have an abundance of precursor, there would be a loss of hormone as the plant remained in the dark (**3**). Unquestionably, the hormone is indirectly dependent upon photosynthesis for its production; the longer a plant is in the dark, the less it will have, unless there is a large supply of precursor which can be transformed into the active hormone. The writer has no information on the amount of precursor in Nicotiana, and it can only be surmised that it is low.

Influence of temperature on hormone content in green plants

In making comparative studies of growth hormone, the temperature under which the plants have been growing may vary considerably if the investigation lasts for some time. The extent to which temperature influences the hormone content of the plant under study is, therefore, of considerable importance. In gaining an understanding of hormone action, temperature studies are also valuable in themselves. With these points in mind, the present study was undertaken.

As no temperature-controlled greenhouses have been available, the study was made during the winter, when the low temperature could be obtained quite readily by shutting off the radiators and opening the windows the proper amount. However, it has been impossible to keep the temperature constant; a variation of several degrees has been the rule, and on sunny days the variation was likely to be more. In most instances there has been a difference in temperature of approximately eight degrees in the two rooms; very rarely has the maximum of the lower temperature been higher than the minimum of the higher temperature during an experiment, though this has happened. The time of the year when these experiments were made is dark and cloudy in Ann Arbor, and this may have produced results different from those that would have been obtained if there had been an abundance of light.

TABLE III

Growth hormone content in tomato plants subjected to different temperatures

Exp.	Date	Curvature of Avena coleoptile produced by plants in			Temperature	Size of plant
		Warm room	Cold room			
					°C.	
266	2/14/44	7.40	5.2 (4)*	5.4 (5)	18–21; 12–15	30–40 in.
267	3/ 6/44	5.25	3.2 (25)	.....	18–22; 12–16	Same plants as in 266
268	3/13/44	8.80	7.9 (3)	.....	18–22; 12–16	4–5 feet
269	3/20/44	7.80	9.1 (1)	.....	18–24; 12–15	20–27 in.
270	3/27/44	7.10	9.0 (1)	.....	18–24; 10–20	18–24 in.
271	4/ 3/44	6.70	5.7 (1)	10.0 (2)	19–24; 13–19	About 24 in.
274	4/24/44	5.40	6.2 (23)	.....	18–30; 15–20	Same plants as in 271

* The number within parentheses after the curvature denotes number of days the plants were exposed to this temperature.

Tomato plants were used in all but two experiments, in which two- to three-week-old corn plants were used.

In table III are given experiments run over a period of two and a half months, using two different lots of tomato plants. In the first three experiments, older tomato plants were used, and here the plants exposed to a lower temperature had consistently less hormone than the plants kept in the warmer compartment. In the last four experiments, younger plants were used, and here almost as consistently the plants exposed to the lower temperature had a higher hormone content. In each lot of plants within an experiment the same amount of material was used, and the dilution was the same. Parts of an experiment are, therefore, comparable; but different experiments are not.

The cause of this difference is rather obscure. It does not seem possible that one group of plants, started a few weeks earlier than another, all from the same package of seeds, would behave entirely differently. Yet no external factors seem to vary enough to account for the difference in the results. It was thought that a greater amount of light in the later experiments might have increased photosynthesis or some other activity that would counterbalance effect of temperature; but a check on the weather for the weeks in question shows actually slightly less sunshine in late March and April than during the preceding weeks. One can only make the inadequate statement that the internal reactions were such that the net result was an increase in the hormone content when the plants were exposed to a lower temperature.

In order to gain more information and somewhat clarify the situation, another series of experiments was run in February of 1945. Tomato plants only slightly larger than the smaller ones in the preceding experiments and young corn plants were used. In these experiments the tomato plants were moved from a compartment of medium temperature to rooms of lower and higher temperatures to obtain, if possible, the influence of an increase as well as a decrease in temperature. The corn plants were exposed to the low temperature for varying lengths of time. Table IV gives the results. Again the lower temperature induced a decrease in hormone, and after a few days in the cold the corn plants showed no hormone, which merely means the concentration was too low to show up under the experimental conditions. Experiment 324 is very interesting as it shows an increase of hormone with an increase in temperature as well as a decrease with the lowering of the temperature. Experiments 271, 327, and 328 bring out something else; namely, that an exposure of one day to low temperature decreases the hormone, which then increases so that on the second day there may even be more than in the plants at the higher temperature. No growth studies have been made, but it is likely that at the lower temperature the hormone is used up in growth relatively more rapidly than it is formed, during the first day when growth rate may be high; later, however, the hormone formation may be somewhat higher than its use. In his extensive study of plant growth under controlled conditions WENT (**18**) finds "There is no correlation between day or night temperature and auxin content." His temperatures were 20° and 26.5° C.

TABLE IV

GROWTH HORMONE CONTENT IN PLANTS SUBJECTED TO DIFFERENT TEMPERATURES

EXP.	CURVATURES OF AVENA COLEOPTILES PRODUCED BY PLANTS IN						TEMPERATURE	KIND AND SIZE OF PLANTS
	WARM ROOM		COLD ROOM					
							°C.	
324	16.3 (2)*	8.2 (1)	3.4 (1)	2.8 (2)	.....	.....	18–29; 10–18	Tomato, 23–28 in., in blossom
326	13.5	.....	7.6 (9)	.....	.....	.....	18–40; 10–15	Same plants as in 324
327	13.2	.....	2.9 (1)	5.0 (2)	.....	.....	18–40; 12–19	Corn, 19 days old
328	8.7	.....	5.2 (1)	9.5 (2)	0 (4)	0 (6)	20–26; 12–19	Same plants as in 327

* The number within parentheses after curvature denotes number of days the plants were exposed to this temperature.

It has been the writer's experience that plants grown either in the greenhouse or outdoors during the summer have a higher hormone content than plants in the greenhouse during the winter, a difference not likely to be due to light conditions but to differences in temperature.

Influence of the parasite Cuscuta on hormone of the host plant

Whenever dodder penetrates the host tissue, there is a swelling as a result of increased growth activity. To see whether there was an increase in the hormone concentration coincident with this increase in growth caused by the parasite, *Cuscuta polygonorum* was allowed to attack plants. It did not grow on many of the plants supplied to it, but it produced an extensive parasitic growth on *Impatiens balsamea* and *Helianthus annuus*. These experiments were conducted in the spring of 1939, before the time the procedure of using the same quantity of plant tissue throughout an experiment had been decided upon, so that it was necessary to calculate the hormone in terms of indoleacetic acid equivalent (**16**); but as comparisons are made only between parts of the plant used on the same day, the error should not be serious. The material was frozen with dry ice before being ground, but the tissue was not boiled to destroy any enzymes. It has been shown by the author (**7**) that the amount of hormone extracted for the first 24 hours with ether from a plant finely ground is not very different from the total hormone obtained when the enzymes have been destroyed by boiling; i.e., during a period of twenty-four hours very little precursor is hydrolyzed to hormone in ether at a temperature of 15° C. Repeated extractions over several months were made, but as the first 24-hour extraction removed all the free auxin (**7**) from the plant material only these data will be used.

In the sunflower experiment, old and young stems infected and not infected and also the Cuscuta vine itself not yet in contact with the plant were used. The infected and much enlarged stem near the base of the sunflower plant contained 3.34 μgm. per kg., a similar uninfected portion contained 2.84 μgm. per kg.; the Cuscuta stem which had its haustoria imbedded in a younger part of the stem contained 5.24 μgm. per kg., while the sunflower stem to which it was attached contained only 3.96 μgm. per kg.; the young parasite not yet attached to the host contained 1.22 μgm. per kg. With Impatiens the same relationship between plant part and auxin content held as for the sunflower, but the amount of hormone was less. It is evident that the parasitic combination increases the auxin so that the infected stem has more auxin than the uninfected; the parasite stem which has been in union with the host has the most hormone; and the young parasite not yet united with the host has the least. It is not possible to say with certainty that the parasite produces the hormone; it can certainly be stated, however, that the attack on the host plant by the parasite produces an increased amount of hormone and that continued extractions of the uninfected stem and the unattached parasite give a total precursor and hormone content of

6.05 μgm. and 35.36 μgm. per kg., respectively. From this one may argue that when the parasite makes organic union with the host, its precursor is hydrolyzed more actively and the hormone flows into the host to increase there the rate of growth. This line of reasoning is perhaps too far-fetched, and it might be better to say that the union of the parasite with the host sets off reactions which increase the hormone in both the host and the parasite.

THIMANN (**13**) considers that the nodules on leguminous roots are the result of auxin production by the Rhizobium spp. which infect their roots. On the other hand, other investigators (**10, 11**) have been led to believe that the crown gall formed by *Phytomonas tumefaciens* is not the result of growth hormones produced by the parasite. Thus we see that there is a controversy as to the part played by growth hormones in parasitism. The present investigation definitely favors the view that when the parasite (Cuscuta) invades the host tissue there is an increase in hormone content which then increases the cell division and produces the typical enlargement in the host plant.

INFLUENCE OF CHLOROPHYLL ON AUXIN CONTENT IN CORN

When the influence of light on hormone was investigated, corn was germinated in the greenhouse and in the darkroom connected to the greenhouse.

TABLE V

AUXIN CONTENT OF GREEN AND ETIOLATED CORN SEEDLINGS

EXP.	DATE	CURVATURE IN AVENA COLEOPTILES PRODUCED BY PLANTS	
		GREEN	ETIOLATED
297	8/ 7/44	7.9	16.4
301	8/14/44	5.1	14.7
303	8/29/44	0.0	4.3

The plants grown in the darkroom were, of course, etiolated; because it was realized that they had not only been subjected to the lack of light but were also without chlorophyll, these plants were not used in the study of the effect of light. They were, however, compared with those grown in the light, and it was found that the etiolated plants contained much more hormone than the green plants (table V).

Because of the two-factor variable it was not possible to explain the large hormone content in these chlorophyll-less plants, but some earlier experiments with albino corn were recalled. In these experiments (exps. 255, 262, table VI) the albino plants also had a much higher hormone content than the green plants. Further experiments with other segregations gave similar results.

How chlorophyll functions to decrease the hormone has not been investigated. Not enough albino seeds have been available to grow plants in the dark and in light. The most obvious explanation is that chlorophyll acts as a photosensitizer, and the exposure to light thus destroys some of the hor-

TABLE VI

AUXIN CONTENT IN GREEN AND ALBINO CORN SEEDLINGS

EXP.	DATE	CURVATURES IN AVENA COLEOPTILES PRODUCED BY PLANTS		REMARKS
		GREEN	ALBINO	
255	11/24/43	3.3	12.2	Mains' corn
262	1/12/44	9.1	18.7	Mains' W18 and W18 (?)
304	11/ 6/44	0.0	5.9	Emerson's 43–85–39 (yellow)
306	11/15/44	0.3+	10.5	Emerson's 43–85–15 (white)
310	11/27/44	3.7	15.6	Mains' corn

mone produced by the plant. The greater hormone content in the etiolated plants may be partly responsible for the greater growth of plants in the dark; however, the albino plants grown in the light are usually slower in developing than the green plants from the same culture.

COMPARISON OF THE AMOUNT OF HORMONE IN DWARF AND TALL CORN PLANTS

In 1935, and later in 1938, VAN OVERBEEK (**14, 15**) considered that dwarf corn was dwarf because the plants produced less and destroyed more hormone than did the tall plants. During the past year the writer has had available dwarf and tall corn seedlings produced from the same ear. Plants about three weeks old from a number of different segregations have been used.

The writer would hesitate to say that the dwarf seedlings have more hormone than the tall seedlings; he would, however, be justified in saying (from these three experiments, which represent plants from at least eight different crosses) that they have at least as much hormone as the tall plants. In another paper in preparation, the matter of inhibitors will be discussed. It may be that the relation between dwarfness and low hormone content found by VAN OVERBEEK was just a coincidence and not cause and effect.

INFLUENCE OF AGE OF AN ORGAN UPON ITS HORMONE CONTENT

In several experiments in which tomato plants 6 to 8 feet tall were used, leaves from different regions were compared as to the amount of hormone

TABLE VII

HORMONE IN DWARF AND TALL CORN PLANTS OBTAINED FROM THE SAME PARENTS

EXP.	DATE	CURVATURE IN AVENA COLEOPTILES PRODUCED BY PLANTS	
		TALL	DWARF
311	11/29/44	6.6	10.6
318	1/ 8/45	9.0	6.3
325	1/31/45	1.2	5.7

they contained. Young leaves near the tip of the plant were always the richest in hormone, and the lowest leaves were the poorest even when they still appeared perfectly healthy. This finding led to a study of the influence of the age of a plant and the position of the growing region on the auxin content of a particular plant. Tips of the main stem with a few young leaves were taken from plants only 12 inches tall and from plants 40 inches tall (a difference in height which means about 2 to 3 months difference in the age of the plant). From the group of the older plants the tips of branches also were collected and examined separately. Experiment 155 will suffice to illustrate the results. The concentration of hormone in the tips of the 12-inch plants was 6.5 μgm. per kg.; in the apical tips of the 40-inch plants it was 3.8 μgm. per kg.; and in the tips of the lateral branches of the same plants it was 3.5 μgm. per kg. Thus it is shown that younger plants have more hormone in the actively growing region than do older plants, and that all growing regions on the same plant have the same amount of hormone. The latter point should be investigated more fully.

Distribution of growth hormone in the flower cluster of the tomato

In parthenocarpic work with chemicals it has always been least difficult to induce fruiting in the first blossom of a cluster of the tomato. The question as to the relative amount of hormone in the different buds has been raised many times. Not only does the first bud set most readily, but it invariably produces the largest fruit. Gustafson and Houghtaling (9) have shown that there is a relation between the size of the ovary in the flower and the size of the fruit set, and that the ovary in the first flower in a cluster is the largest. Is food, as these investigators thought, the only factor in the production of a large ovary in the first flower, or is there also a hormonal difference?

TABLE VIII

Hormone content of the ovaries from flower buds situated in different positions in a flower cluster. The hormone concentration is given in indoleacetic acid equivalents of micrograms per kilogram

Exp.	Date	Location of bud in cluster		
		1	2	3, 4
124	7/19/41	50.2	18.8	11.7
177	6/22/42	12.0	7.5	.....
180	6/29/42	27.7	11.6	.....

Flower buds one and two were always obtained from the same cluster and buds three and four, when used, came from an older cluster; i.e., one in which buds one and two had already opened. The data show that there is a difference in hormone content of the ovaries from flower buds located in different positions in a cluster. The first one formed has the highest concen-

tration and the second the next. The readiness with which the first flowers in a cluster set fruit may be connected with this high hormone content although further development does also depend on such nutritional factors as food and water.

Discussion

In this series of experiments, growth hormone concentration tends to correlate with rate of growth. That is, those conditions like rich soil, high temperature, and moderate intensity of light, which we know favor vegetative growth, are also favorable to the production of growth hormone. Even intumescences produced by parasites like Cuscuta can be considered as a product of the high growth hormone concentration obtained in the host as a result of the parasitic attack.

Like the external factors, internal factors also have an influence on hormone production. In previous experiments (**8**) the author has related fruitfulness in Montmorency cherry to the amount of hormone present in the flower buds; in the present investigation this idea is further strengthened by finding that those buds in the inflorescence of the tomato which most readily produce fruits also have a high hormone content. This statement does not minimize the importance of food, water, enzymes, or accessory growth factors; it merely emphasizes the importance of the hormone.

Differences in chromosome number have previously (**8a**) been shown to be associated with hormone production; in this series of experiments it is shown that chromosomal differences may also influence hormone content of a plant. Corn plants lacking the ability to produce chlorophyll produce, or at least contain, more growth hormone than those plants that produce chlorophyll. It remains to be determined whether or not light is a factor here. Perhaps chlorophyll is an agent through which light destroys some of the hormone produced by the green plant. The experiments with the etiolated corn plants seem to warrant such an assumption, but only by growing albino and chlorophyll-producing plants in the dark can this be determined. Not enough seeds have been available to do this at the present time. Whatever the mechanism, the fact remains, that corn plants unable to produce chlorophyll contain more hormone than plants that do produce chlorophyll.

It is a well-known fact that as a plant grows older its physiological activities change and that young shoots of the same species, but from individuals of different age, may respire or photosynthesize at different rates and that they are even able to strike roots at different rates. The same is seen to be true for growth hormones; shoot tips from young plants contain more hormone than those from older plants. It seems to the writer that whatever else this series of experiments may have done it ties hormones more closely to physiological phenomena and makes them more definitely members of the family.

Summary

It has been found that poor mineral nutritive conditions, low tempera-

ture, and high light intensities lower the growth hormone content in plants investigated (tomatoes and corn).

Stems of *Helianthus annuus* and *Impatiens balsamea* parasitized by *Cuscuta polygonorum* have a higher growth hormone content than stems not so parasitized.

Corn seedlings devoid of chlorophyll, either because they have been grown in the dark or because of their genetic constitution, have more growth hormone than do similar plants with chlorophyll.

As a part of a plant, or the plant as a whole, ages its growth hormone content becomes less.

Flower buds formed first in an inflorescence of the tomato have more hormone and are more likely to set fruits than those formed later.

The dodder seeds were obtained from Dr. H. L. Dean at the State University of Iowa, Iowa City, Iowa.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

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THE LACK OF SCION EFFECT ON ROOT QUALITY OF *DERRIS ELLIPTICA*[1]

MERRIAM A. JONES AND WILLIAM C. COOPER

Introduction

In rotenone-bearing crops, such as *Derris elliptica* (Wall.) Benth., in which the root is valued for insecticidal purposes, the yield of root as well as the rotenone content is important. Obviously yield could not be sacrificed to obtain high rotenone percentage, nor could high yield compensate for low rotenone content. A maximum yield of root of uniformly high rotenone content may be considered as a goal.

Of the several types of *D. elliptica* available at this station, one, the St. Croix variety, is characterized by a high yield of root markedly low in rotenone. Another, Changi No. 3, is characterized by a lower yield of root which is relatively high in rotenone. A third variety, the Sarawak Creeping, occupies an intermediate position with regard to rotenone content.

The extent to which grafting may modify the yield and rotenone content in Derris roots has never been investigated. The possibility of increasing the rotenone content of the more vigorous St. Croix roots by grafting on scions of the Changi No. 3 seemed promising. Likewise, St. Croix tops on Changi No. 3 roots might increase the yield of roots of the latter. An experiment testing these premises is here reported, the results of which, although negative with regard to the anticipated scion effect, are of interest.

A hypothesis under test in this experiment concerned the possibility of the leafy part of the plant supplying some factor, perhaps an intermediate compound, similar to but simpler than rotenone, which was necessary for the completion of the synthesis of rotenone in the roots. This was suggested by the fact that compounds of the rotenone group are chemically related to compounds of the flavone group, of which at least two occur in the leaves of derris.[2] Rotenone itself (I) can be considered as an isoflavanone nucleus (II) in which a methoxyl group on 6′ is condensed with a hydroxyl group on 2 to result in a bridge. On 7 and 8 there is a pentose residue and on 3′ and 4′ methoxyl groups.

That compounds related to rotenone exist in the root in glycosidic combination (probably acylated) is indicated by the fact that derris resin must be hydrolyzed by mild alkali treatment before crystalline deguelin can be isolated.

Methods

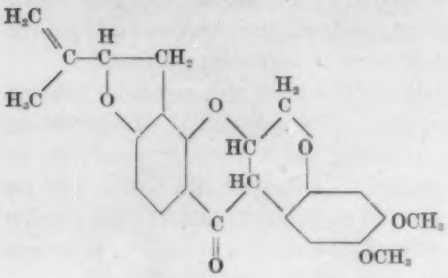
Reciprocal grafts of high-rotenone Changi No. 3 and low-rotenone St. Croix were made by whip grafting leafless stem cuttings of the 2 varieties.

[1] This work was done at the Federal Experiment Station, Office of Experiment Stations, Agricultural Research Administration, U. S. Department of Agriculture, Mayaguez, Puerto Rico, and was a cooperative project between this Station and the Office of Foreign Agricultural Relations, U. S. Department of Agriculture.

[2] Jones, M. A. Unpublished data.

Control grafts consisting of each type grafted on itself were made at the same time. Ungrafted control cuttings of each variety were also included in the experiment making a total of 6 treatments each consisting of 10 grafts. The cutting material used was taken from leafy vines with mature brownish bark. Since all material of each type was taken from an individual plant, each resulting group of plants was a single clone.

The grafts were prepared in the laboratory and tied with string, no wax or bandage being used. Cuttings were placed in a sash-covered sand bed in the greenhouse at high humidity for 6 weeks; during this time the unions healed and the stocks rooted. They were then transplanted to nursery beds in the field, each row constituting one treatment. During the time the plants were growing in the field as well as in the propagating frame, the spacing was 2 inches between plants in rows 4 inches apart. The unions were always kept clear of the soil in order to avoid separate rooting of the scion.



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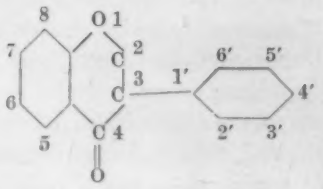

II

Eleven months after grafting, the tops were removed and the roots of each plant excavated as completely as possible. The roots were then divided into two size groups, those smaller than 5 mm. diameter and those 5 mm. and over.

To obtain a measure of quality of the separate small samples, the dry root was weighed and ground through the 20- and 60-mesh sieves of a micro Wiley mill, and colorimetric determinations were made for rotenone plus rotenoids in each sample (**1**), after which suitable composites were made for an additional colorimetric test and rotenone analyses by the A.O.A.C. method (**2**).

A similar experiment was performed simultaneously in which grafts were made between Sarawak Creeping and St. Croix.

Results

The roots of the St. Croix stocks tended to be of large diameter, some over 10 mm., with few fine roots. The root system of the Sarawak Creeping variety stock tended to be larger than that of Changi No. 3 and considerably more branched than that of the St. Croix. At the time of harvest it was noted that the bark of some roots of the Changi variety contained a reddish pigment whether the scion was Changi No. 3 or St. Croix.

Chemical analyses of the roots, summarized in table I with the survival and yield data, show no effect of scion on yield of root or on rotenone content.

TABLE I

EFFECT OF GRAFTING VARIETIES OF *Derris elliptica* ON THE YIELD AND QUALITY OF THE ROOT

GRAFTS	PLANTS SURVIVING	YIELD, DRY ROOT PER PLANT		COLORIMETRIC, ROTENONE PLUS ROTENOIDS				ROTENONE*	RATIO ROTENONE TO ROTENONE PLUS ROTENOIDS
		RANGE	AVERAGE	CONTENT		PER PLANT			
				RANGE	WEIGHTED AVERAGE	RANGE	WEIGHTED AVERAGE		
scion/stock	*no.*	*gm.*	*gm.*	%	%	*gm.*	*gm.*	%	
Changi No. 3 ungrafted	10	0.2 – 6.95	3.05	8.9–12.6	11.0	0.03–0.87	0.33	5.7 and 6.9	0.52
Changi No. 3/Changi No. 3	5	0.8 –11.15	4.47	3.2– 9.9	8.5	0.03–1.10	0.38	4.5	0.55
St. Croix/Changi No. 3	10	0.5 – 8.4	3.30	7.3–11.6	9.4	0.05–0.89	0.31	5.2 and 5.9	0.56
St. Croix ungrafted	10	1.85–26.45	8.82	1.2– 5.9	2.9	0.05–0.54	0.26	0.9 and 1.4	0.45
St. Croix/St. Croix	8	1.4 –15.5	5.13	2.4– 4.2	2.9	0.05–0.38	0.15	1.1	0.34
Changi No. 3/St. Croix	9	0.45– 8.85	3.37	2.4– 4.6	3.5	0.02–0.27	0.12	1.1 and 1.5	0.35
Sarawak Creeping/St. Croix†	3	3.65–11.8	6.48	1.1– 4.1	2.1	0.13–0.15	0.14	1.0	0.45
St. Croix/Sarawak Creeping†	3	3.0 –13.25	7.87	4.0– 4.9	4.3	0.12–0.53	0.33	2.7	0.57

* Two figures are reported for those treatments of which there was sufficient material to prepare two samples for rotenone analysis.
† The controls of these two treatments did not survive.

Another striking feature in the data was the wide variation among plants of the same clone, especially with regard to quality as measured by the colorimetric test or rotenone content. No correlation could be found between quality and yield in any of the treatments.

The ratio of rotenone to rotenone plus rotenoids in the St. Croix root material was low, about 0.35 to 0.45; that of the higher quality Changi No. 3 and Sarawak Creeping had a ratio of 0.50 to 0.55.

Discussion

It was not considered necessary to transplant the experimental material to the field to await maximum root and top development because the effect of scion on stock is a definite one; that is, either there is an effect or there is none. Any increase or decrease of rotenone in the stock caused by the grafted scion would be apparent in the young as well as in the more developed plant. In this experiment rotenone was found in comparatively large amounts in roots of Changi No. 3 and in much smaller concentrations in roots of the St. Croix regardless of tops used. Therefore, although growth took place in these plants and the scion obviously furnished food to the stock, no effect on rotenone content could be demonstrated. The grafted and ungrafted plants contained rotenone in concentrations characteristic of the stock. Thus it appears that the leafy top of the plant probably does not control production of rotenone by furnishing a hypothetical precursor which would be transported to the roots and deposited there as rotenone. The synthesis of rotenone would therefore appear to be confined principally to the root system. This phenomenon resembles that found in reciprocal grafts among tomato, Datura, and tobacco (**3**) in which it was found that the root was the center of synthesis of some plant poisons.

Summary

Grafting between high- and low-rotenone varieties of *Derris elliptica* (Wall.) Benth. showed that there was no effect of top on root as far as yield of root and percentage of rotenone were concerned. This indicates that the root system is not dependent on the top for a unique precursor of rotenone.

The authors are indebted to Mr. Caleb Pagan, employed by the War Emergency Program of the Government of Puerto Rico, for his aid in part of the chemical analyses.

Federal Experiment Station
Mayaguez, Puerto Rico, and
Subtropical Fruit Field Station
Orlando, Florida

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EFFECT OF AUXINS ON THE ACTION OF DIASTASE *IN VITRO*

H. C. Eyster

(WITH TWO FIGURES)

It has been shown by Eyster (**2**) that indole-3-propionic acid both retards the action of isolated diastase on soluble starch and accelerates the action of diastase associated with charcoal by releasing the adsorbed diastase from the charcoal. In this paper the actions of indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid are compared both in their effects on the action of isolated diastase and in their effects on the action of diastase adsorbed upon activated charcoal.

Methods and materials

The technique used here is the same as that described by Eyster (**2**), wherein the enzyme, diastase of malt Merck (medicinal, U.S.P. IX), was adsorbed upon particles of activated charcoal. The effectiveness of diastase as a hydrolytic agent for soluble starch was measured by determining the time required to digest a given amount of soluble starch past the last iodine-staining stage.

Norit A was the type of activated charcoal used. It was obtained by the University of South Dakota Chemistry Department from the Pfanstiehl Chemical Company in Waukegan, Illinois. Norit by definition (**3**) is a purified charcoal made from birchwood and is used to decolorize and deodorize syrups, oils, or pharmaceutical products. Inasmuch as the Norit A showed variations in adsorptive capacity with changes in the moisture content of the atmosphere, it was dehydrated by placing it in an oven at 107° C. for 24 hours and then stored in a desiccator with dry $CaCl_2$. Since there was the possibility that the adsorptive capacity of the charcoal may be affected by CO_2 content of the atmosphere, KOH was also placed in the desiccator. The temperature of the solutions was always kept close to 25° C. by the use of a water bath. All of the special chemical compounds used in this research were purchased from Eastman Kodak Co., Rochester, N. Y.

Results

To test the effect of the auxins on the action of isolated diastase 25 and 50 p.p.m. of each auxin were used in separate mixtures. The control mixture contained 50 ml. of 1 per cent. soluble starch, 49 ml. of distilled water, and 1 ml. of 1 per cent. diastase of malt. The mixtures having 25 p.p.m. auxin were made up of 50 ml. of 1 per cent. soluble starch, 5 ml. of a solution containing 500 p.p.m. auxin, 44 ml. of distilled water, and 1 ml. of 1 per cent. diastase of malt. The mixtures having 50 p.p.m. auxin were prepared by taking 50 ml. of 1 per cent. soluble starch, 10 ml. of a solution containing 500 p.p.m. auxin, 39 ml. of distilled water, and 1 ml. of 1 per cent. diastase of malt.

The time required for the digestion of the soluble starch past the last iodine-staining stage, and the pH of each mixture was determined. The rate of digestion was determined by the simple I_2KI test, using a dilute solution. A glass electrode pH meter was used to determine the pH of each mixture.

The results are recorded in table I giving the effect of the respective auxins on enzyme action proper. Indole-3-propionic acid has the least retarding effect on the action of isolated diastase. It is followed in the order of effectiveness by indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid. Alpha-naphthalene acetic acid is the most active of the four auxins used. If the control is arbitrarily assigned the value of 100 for its digestive rate, the digestive rates of the mixtures with 25 p.p.m.

TABLE I

EFFECT OF FOUR DIFFERENT AUXINS ON THE ACTION OF ISOLATED DIASTASE; I.E., DIASTASE NOT ASSOCIATED WITH ACTIVATED CHARCOAL

MIXTURES	TIME REQUIRED FOR DIGESTION PAST LAST IODINE STAINING STAGE	PERCENTAGE OF CONTROL*	pH
	min.	%	
Control	125	100	5.9
Indole-3-propionic acid, 25 p.p.m.	132	95	5.5
Indole-3-butyric acid, 25 p.p.m.	150	83	5.2
Indole-3-acetic acid, 25 p.p.m.	165	76	5.3
Alpha-naphthalene acetic acid, 25 p.p.m.	175	71	4.7
Indole-3-propionic acid, 50 p.p.m.	145	89	5.0
Indole-3-butyric acid, 50 p.p.m.	160	78	4.9
Indole-3-acetic acid, 50 p.p.m.	170	74	4.7
Alpha-naphthalene acetic acid, 50 p.p.m.	185	68	4.5

* Time for control divided by time for experimental test, expressed in percentage.

of indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid are calculated to be 95, 83, 76, and 71, respectively. The effects of 50 p.p.m. of each of the auxins are greater.

If pH and the rapidity of digestion are plotted as in figure 1, it can be seen that there is a direct relationship. The sole cause of the difference in the effect of the four auxins on isolated diastase seems to result from differences in their hydrogen ion concentration. Corroborating evidence was obtained by using very dilute solutions of acetic acid to produce a series of pH levels and then to test the rapidity of diastase action at those pH levels. The lower the pH, the slower was the diastatic activity on soluble starch. Buffer solutions were not used to stabilize pH because buffer salts are not neutral in their effects on enzyme action.

To determine the effects of auxins on the action of diastase adsorbed upon activated charcoal, the control mixture contained 50 ml. of 1 per cent. soluble starch, 45 ml. of distilled water, 5 ml. of 1 per cent. diastase, and 1 gm. of Norit A; mixtures with 25 p.p.m. auxin had 50 ml. of 1 per cent. soluble

starch, 5 ml. of a solution containing 500 p.p.m. auxin, 40 ml. of distilled water, 5 ml. of 1 per cent. diastase, and 1 gm. of Norit A; and mixtures with 50 p.p.m. auxin had 50 ml. of 1 per cent. soluble starch, 10 ml. of a solution containing 500 p.p.m. auxin, 35 ml. of distilled water, 5 ml. of 1 per cent. diastase, and 1 gm. of Norit A. In preparing the mixtures, all of the ingredients except diastase and Norit were mixed; the diastase was then added. The mixtures were shaken a bit and the Norit added quickly with enough shaking to thoroughly mix it with the other constituents. Every one-half

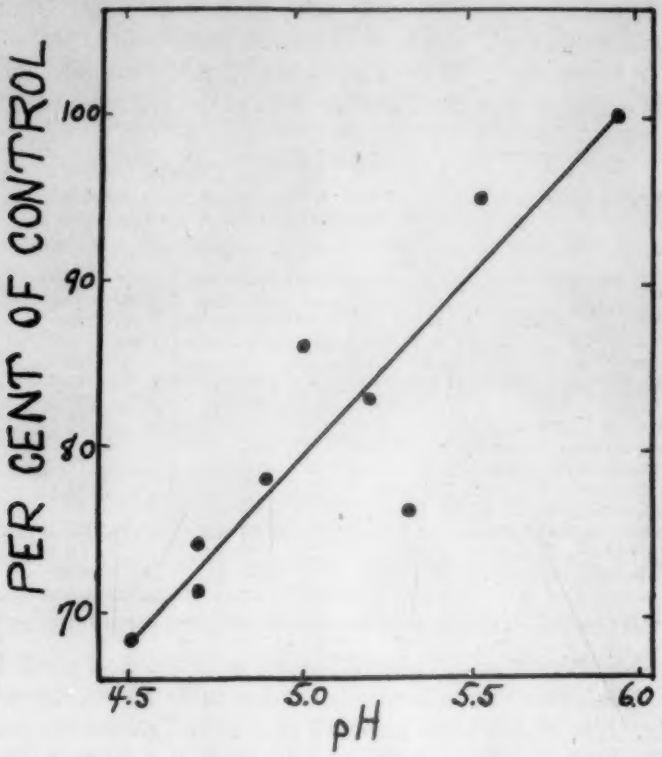

FIG. 1. Interrelation of pH and rate of digestion by diastase. In the range between pH 4.5 and 6.0, as the pH increases the rate of digestion increases by a seemingly constant acceleration.

hour the solutions were shaken, and 2 ml. of each mixture were filtered through two thicknesses of fine textured filter paper and tested with one to several drops of a dilute solution of I_2KI. One drop was used during the initial stages of the experiment, and two to five drops were used later on. The data are presented graphically in figure 2. The results indicate the same order of effectiveness as was obtained in the previous experiment. Indole-3-propionic acid is the least active and alpha-naphthalene acetic acid is the most active. The periodic tests every 30 minutes indicated that, at the start, the least active auxin appeared the most active, and the most active auxin appeared the least active. At the start, it appears that most of the

diastase is adsorbed and thereby inactivated. The small portion which is still free to act would then be impeded least by the least active auxin. Digestion in the control is slowest and possibly is impeded because the diastase is more thoroughly adsorbed there than in mixtures containing auxins. Auxins release the diastase from the activated charcoal. The interaction of auxins and diastase adsorbed on charcoal apparently involves two phases, accounting for a change in the rate of digestion. At first the auxin interaction is dominated in a phase characterized by the retardation of enzyme action proper of the unbound diastase. Later the auxin interaction is dominated in a phase characterized by the release of bound diastase and a consequent acceleration in diastase activity. The slowness of the mixture with indole-3-propionic acid, at the end, is probably due to its extreme ineffectiveness in releasing diastase from the activated charcoal and its simultaneous retardation of enzyme action proper. It appears that the release of diastase by auxins consists of a displacement of auxins on the charcoal surface by hydrogen ions. The least active auxins appear to displace very little diastase leaving an abundance of free auxin to retard the small amount of free enzyme.

The tests with iodine, to show the relative amounts of soluble starch still undigested, were very consistent, clear-cut and definite. While the total time required for digestion, past the last iodine-staining stage in the four auxin and one control mixtures, may not be significant, there was a consistent and orderly trend in the course of digestion in each solution.

The pH of all of these charcoal mixtures regardless of the auxin present is the same—pH 8.2. Control solutions without any auxin also have the same pH. It is the belief of the author that hydrogen ions here too play a dominant influence in the release of auxins from the surface of charcoal particles and that there are pH contours closely surrounding the charcoal particles. While the pH of the free fluid between the charcoal particles is 8.2, the adsorbed layers on the surface may have lower and lower pH's with the lowest pH on the immediate surface. The pH on the immediate surface is expected to vary with the auxin used, and this, if true, would explain the variation in the release of auxin by displacement by various auxins and by various concentrations of the same auxin. There is evidence that ions other than hydrogen influence adsorption of charcoal and enzymes (1) and so, with the great diversity of numerous growth substances, the interaction of growth substances and enzymes need not in all cases be explained on the basis of hydrogen ion concentration effects.

The rate at which different auxins release diastase from activated charcoal was determined with another experiment. One gm. of Norit A was mixed with 35 ml. of distilled water and 5 ml. of 1 per cent. diastase and 2½ hours allowed for fairly complete adsorption of the diastase. Immediately at the end of 2½ hours, 50 ml. of 1 per cent. soluble starch, 5 ml. of a solution containing 500 p.p.m. auxin and 5 ml. of distilled water were added. This gave the final mixtures auxin concentrations of 25 p.p.m. To other mix-

tures, 50 ml. of 1 per cent. soluble starch and 10 ml. of a solution containing 500 p.p.m. auxin were added to produce mixtures with concentration of 50 p.p.m. Digestive tests with I_2KI were made on these solutions at the end of 24, 31, 44, 48, and 50 hours. The ones with 25 p.p.m. indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid are of special interest. ' Twenty-four-, 31-, 44-, and 48-hour tests with 25 p.p.m. and 50-hour tests with 50 p.p.m. gave significant results which conform with the results of the preceding experiment.

In the 24-hour test using 25 p.p.m. auxin, the order from greatest amount of digestion to the least was (1) indole-3-propionic acid, (2) indole-3-butyric acid, (3) indole-3-acetic acid, (4) alpha-naphthalene acetic acid, and (5) control. Seven hours later (31-hour test) the order was (1) indole-3-acetic acid, (2) indole-3-propionic acid, (3) indole-3-butyric acid, (4) alpha-naphthalene acetic acid, and (5) control. The 44-hour test had (1) indole-3-acetic acid, (2) alpha-naphthalene acetic acid, (3) indole-3-propionic acid, (4) indole-3-butyric acid, and (5) control; while the 48-hour test had (1) alpha-naphthalene acetic acid, (2) indole-3-acetic acid, (3) indole-3-propionic acid, (4) indole-3-butyric acid, and (5) control. In the 50-hour test using 50 p.p.m. auxin, the order from the greatest amount of digestion to the least was (1) alpha-naphthalene acetic acid, (2) indole-3-acetic acid, (3) indole-3-butyric acid, (4) indole-3-propionic acid, and (5) control.

Discussion

There is much diversity of results in the literature on the comparative effectiveness of auxins on plant growth. This can possibly be explained on the basis of variations of concentrations used, the time factor, and the influence of light in some cases. The more active auxins, *in vitro,* appear to be less effective in greater concentrations whenever the time is comparatively brief. This is clearly indicated in figure 2. The comparative digestive stages in the auxin-diastase-charcoal soluble starch mixtures vary continually, with the less active auxins more rapid at first followed and surpassed by the more active auxins. While there is considerable diversity, the literature contains records in the majority of which the order of comparative effectiveness is the same as that demonstrated in this paper by the charcoal technique. MARMER (6) found that the concentrations causing 50 per cent. reduction in growth of primary root of Marquis wheat seedlings at pH 4.6 were: indole-3-acetic acid, 0.012 mg. per liter; indole-3-butyric acid, 0.055 mg. per liter; and indole-3-propionic acid, 0.250 mg. per liter. The exact position of alpha-naphthalene acetic acid in the series is indicated by MACHT and GRUMBEIN (5). They found that the growth of roots of *Lupinus albus* seedlings was definitely retarded by long exposures to alpha-naphthalene acetic acid, indole-3-acetic acid, and indole-3-butyric acid. The former was the most effective and the latter least effective of the three auxins used. ZIMMERMAN'S (7) data indicate the same order of effectiveness. For total root lengths of Elodea at 16 days using

a concentration of 1 p.p.m., KING (4) found that the order of decreasing magnitude of response was indole-propionic acid, indole-butyric acid, indole-acetic acid, 1-tryptophane, phenyl-acetic acid, control, vitamin B_1, and naphthalene acetic acid.

From the *in vitro* experiments described in this paper, it might be concluded hypothetically that there are two phases in the interaction of auxins on charcoal-adsorbed diastase: phase I, release of enzyme in whole or in part from the charcoal; and phase II, effect on enzyme action proper whereby reactants are adsorbed upon the surface of the enzyme and are made to combine to form end products, apparently because the reactant molecules are subjected to gigantic adsorptive pressures as well as by being

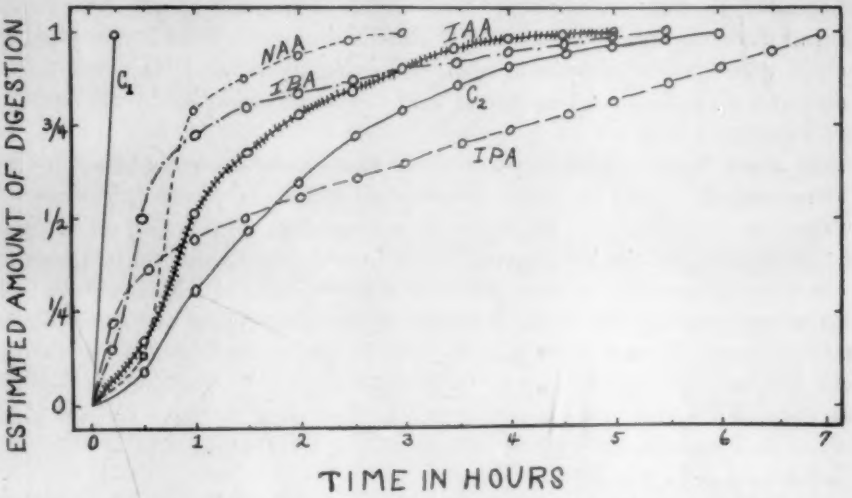

FIG. 2. Effect of auxins on the action of diastase adsorbed upon activated charcoal. The digestion was followed colorimetrically up to and including the last iodine-staining stage at half-hour intervals. C_1, control in the absence of activated charcoal; C_2, control in the presence of activated charcoal; NAA, with alpha-naphthalene acetic acid; IBA, with indole-3-butyric acid; IAA, with indole-3-acetic acid; and IPA, with indole-3-propionic acid.

brought into closer proximity. If the influence of an auxin or factor dominates in phase I, then the action of the enzyme is accelerated because more unbound enzyme is made available, and the factor can logically be called a "growth-promoting factor" or "growth substance." If the influence of the same auxin or factor dominates in phase II, then the action of the enzyme is decelerated, and the factor can be called a "growth-inhibiting factor" or "growth inhibitor." In each phase, adsorption is an important process involved.

The interaction of auxins and diastase on charcoal surfaces may well be the way in which auxins and enzymes interact on the surfaces of cellular colloids.

Summary

The interaction of auxins and diastase of malt was investigated *in vitro*, both in isolated and activated charcoal systems. Indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid were used in concentrations of 25 and 50 p.p.m. at 25° C. In the activated charcoal systems 1 ml. of 1 per cent. diastase was adsorbed upon 1 gram of Norit A. The influence of the auxins on diastase, in isolated systems, was measured by determining the time required to digest a given amount of soluble starch past the last iodine-staining stage. The influence of auxins on diastase in activated charcoal systems was measured by regular half-hour comparative color tests with I_2KI. Auxins retarded the action of isolated diastase and accelerated the action of diastase adsorbed on charcoal in the same order. The order of effectiveness from least active to most active was: indole-3-propionic acid, indole-3-butyric acid, indole-3-acetic acid, and alpha-naphthalene acetic acid. The effectiveness of the auxins was correlated with pH.

It might be concluded hypothetically that there are two phases in the interaction of auxins on charcoal-adsorbed diastase: phase I, release of enzyme in whole or in part from the charcoal; and phase II, effect on enzyme action proper. If the influence of an auxin or factor dominates in phase I, then there is increased enzyme action, and the factor can logically be called "growth-promoting." If the influence of the same auxin or factor dominates in phase II, then there is a decrease in enzyme action, and the factor can just as logically be called "growth-inhibiting." The interaction of auxins and diastase on charcoal surfaces may well be the way in which auxins and enzymes interact on the surfaces of cellular colloids.

DEPARTMENT OF BOTANY
UNIVERSITY OF SOUTH DAKOTA
VERMILLION, SOUTH DAKOTA

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EFFECTS OF IRON ON CERTAIN NITROGENOUS FRACTIONS OF *ANANAS COMOSUS* (L.) MERR.[1]

C. P. Sideris and H. Y. Young

(WITH SIX FIGURES)

Introduction

The effects of ammonium- vs. nitrate-nitrogen in solution cultures on the nitrogenous fractions in the tissues of *Ananas comosus* (L.) Merr. were reported in previous publications (**31, 32**). Data on the effects of plus- or minus-iron cultures supplied with nitrate- or ammonium-nitrogen on the growth and the tissue content of various ash constituents, chlorophyll, acidity, ascorbic acid, and carbohydrates of *A. comosus* were also reported in two former papers (**29, 33**).

This paper is concerned with the effects of plus- vs. minus-iron cultures in association with those of ammonium- or nitrate-nitrogen on the nitrogenous fractions of the tissues of *A. comosus*.

Methods

Methods employed for growing the experimental plants were described in the first paper of this series (**33**) while the procedure for sectioning and preparing them for chemical analysis was reported in earlier publications (**30, 31**). In brief, the plants were grown for 12 months in 4-gallon porcelain containers, then harvested and sectioned for analysis. The nutrient solutions were aerated constantly and changed regularly at 2-week intervals.

The nitrogenous fractions studied were: total nitrogen, sap-soluble inorganic-N, nitrate-N, ammonium-N, sap-soluble organic-N, amide-N, alpha-amino-N, mono-amino-N, basic-N, peptide-N, proteose-peptone-N, and protein-N.

Nitrate nitrogen was determined by the phenoldisulfonic acid method. Total nitrogen, including nitrates, was determined according to the methods of Pucher *et al.* (**26**) and Vickery (**35**).

The procedure for the separation of the different fractions was as follows: The pulp from 50 grams of fresh plant tissues, thoroughly ground in a mortar with quartz sand, was placed in a 100-ml. flask to which was added 1 ml. of glacial acetic acid and water to the mark; the mixture was heated to boiling and then centrifuged or filtered. The centrifugate or filtrate was removed and the residue washed twice with 25 ml. of hot 1 per cent. acetic acid. The centrifugate from both washings was combined; the residue was employed for the determination of insoluble or protein nitrogen. Total nitrogen in the centrifugate after subtraction of nitrate and proteose-peptone nitrogen was classified as soluble organic nitrogen. A 10-ml. aliquot of the

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centrifugate was employed for the determination of alpha amino nitrogen by the Van Slyke method after subtracting ammonium nitrogen (values ranging from 0.002 to 0.020 milligrams per gram of fresh tissue) which was determined by the method of PUCHER, VICKERY, and LEAVENWORTH (**27**).

The centrifugate was treated with 10 ml. of lead acetate (10 gm. of lead acetate in 90 ml. of 1.5 per cent. acetic acid) and allowed to stand overnight. The precipitate was separated from the supernatant liquid by centrifugation and washed with 20 ml. of 80 per cent. alcohol. It was analyzed for nitrogen by the Kjeldahl process, using selenium oxychloride as catalyst. This fraction was designated as the "soluble protein" fraction and was probably identical with the so-called "proteose-peptone" fraction, as evidenced by its precipitation with lead acetate and tannic acid (**23**).

The filtrate or centrifugate from the above operation, containing also the alcoholic washings, was treated with 10 ml. of mercuric acetate (10 gm. of mercuric acetate in 100 ml. of a 2.0 per cent. acetic acid) without removal of the excess lead of the former treatment. The removal of such lead in the form of lead sulfide or lead sulfate, either with hydrogen sulfide gas or with sulfuric acid and precipitation of excess sulfuric acid with $Ba(OH)_2$, was omitted because it removed some nitrogen by adsorption. The mixture was allowed to stand overnight, and the precipitate was removed either by centrifugation or filtration and then washed with 20 ml. of 80 per cent. alcohol. The precipitate was analyzed for nitrogen by the Kjeldahl process as mentioned above. This fraction of nitrogen was classified as peptide (**17**).

The centrifugate or filtrate from the above operation was treated with 3 ml. of concentrated H_2SO_4, and the precipitate, consisting mostly of $PbSO_4$, was removed by centrifugation and subsequently washed twice with H_2O. The precipitate was discarded, and the centrifugate was made to 200 ml., treated with 4 ml. of concentrated H_2SO_4, heated over a boiling water bath for 3 hours, then made alkaline for the distillation of ammonia under the conditions recommended by PUCHER, VICKERY, and LEAVENWORTH (**27**) for amide nitrogen.

After the removal of amide nitrogen the mixture was acidified with H_2SO_4 and treated with phosphotungstic acid for the separation of monoamino-nitrogen from basic-nitrogen as described previously (**30**).

No fractionation of protein nitrogen was attempted because somewhat comparable data had already been obtained in a former study (**31**).

The statistical significance of the difference of the items between the F and O cultures in tables I to V, calculated by CONRAD'S method (**15**) in combination with LOVE'S table (**19**) is reported in table V.

The synoptic expressions introduced in the two former papers of this series to replace the long appellations of the different treatments have also been adopted for this paper. Thus, the "plus-iron" cultures will be designated by F and the "minus-iron" by O. Also, the ammonium-nitrogen series will be designated by A-n and the nitrate-nitrogen by N-n.

Results

Total nitrogen

The amounts of total nitrogen varied in different sections of the plants of the various treatments as reported in tables I to IV. These data show that more nitrogen accumulated in the terminal sections of the leaves and in the apical ones of the stem than in the basal or intermediate sections of these organs. Similar results also were obtained in former studies (**31, 32**). Total nitrogen values were greater in the chlorophyllose sections of the leaves of the A-n than N-n series, also in agreement with other results reported previously (**31**). The higher values of total nitrogen in the stem and in many of the basal sections of the leaves in the N-n than A-n series resulted possibly from accumulations of nitrate-nitrogen which, in accordance with former studies (**31, 32**), is not assimilated readily in the non-chlorophyllose tissues of the plant. However, assimilation of nitrate is very rapid in the chlorophyllose tissues of the leaves.

Absorption of nitrogen by the plants, as indicated by the total nitrogen content of tissues, was greater from the F than O cultures. Moreover, the plants of the F cultures in the A-n series contained greater amounts of nitrogen than those in the N-n series. There was, in this connection, good correlation between amounts of total nitrogen and chlorophyll in the leaves (**29, 33**). The absorption and the subsequent assimilation of nitrate was greater from the F than O cultures in the N-n series which suggests an increased physiological activity in the F cultures, presumably on account of greater chlorophyll contents resulting from the presence of ample amounts of iron. The differences in the amounts of total nitrogen between the F and O cultures in the A-n series were small, and they were possibly induced by the relatively small differences in the physiological activity of these plants on account of the small variation in chlorophyll content.

Total organic nitrogen

Total organic nitrogen, comprising all fractions except the nitrate- and ammonium-nitrogen, was generally higher in the F than O cultures. The differences in organic nitrogen between the F and O cultures were greater in the A-n than in the N-n series as reported in tables I to IV. Organic nitrogen in the A-n series was not consistently greater in the F than O cultures in all sections of the leaves and stem. However, the data in table V show that the differences in the leaves were highly significant between the F and O cultures.

Organic nitrogen values in leaves and roots were inversely proportional, being high in the roots of cultures containing low amounts in the leaves, and vice versa. For example, the organic nitrogen values of the roots were greater in the O than in the F cultures, being 95.0 $\left(\frac{1.288-0.662}{0.662}\right)\times 100$ and 25.7 $\left(\frac{1.176-0.936}{0.936}\times 100\right)$ per cent. for the N-n and A-n series, respec-

TABLE I

FRACTIONS OF INORGANIC-, SOLUBLE ORGANIC- AND PROTEINACEOUS-NITROGEN IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR., GROWN IN MINUS-IRON SOLUTION CULTURES SUPPLIED WITH NITRATE-NITROGEN

PLANT SECTIONS	MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE										
	INORGAN.	SOLUBLE ORGANIC					PROTEINACEOUS			TOTAL	
	NITRATE	PEPTIDE	AMIDE	MONO-AMINO	BASIC	TOTAL	PROTEOSE AND PEPTONE	PROTEIN	TOTAL	ORGANIC	GEN-ERAL*
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves											
Old (B) 1+2 (base)	0.177	0.018	0.046	0.140	0.034	0.238	0.040	0.544	0.584	0.822	0.999
3	0.015	0.024	0.088	0.108	0.030	0.250	0.060	0.738	0.698	1.048	1.063
4	0.000	0.038	0.058	0.160	0.060	0.316	0.048	0.884	0.932	1.248	1.248
5 (tip)	0.000	0.036	0.066	0.180	0.048	0.330	0.052	1.004	1.056	1.388	1.388
Mature (C) 1 (base)	0.364	0.006	0.032	0.184	0.032	0.254	0.018	0.376	0.394	0.648	1.012
2	0.318	0.012	0.036	0.136	0.030	0.214	0.028	0.526	0.554	0.768	1.086
3	0.029	0.018	0.026	0.108	0.012	0.164	0.036	0.842	0.878	1.042	1.071
4	0.009	0.040	0.060	0.172	0.042	0.314	0.046	1.004	1.050	1.364	1.373
5 (tip)	0.000	0.048	0.070	0.184	0.068	0.370	0.066	1.164	1.230	1.600	1.600
Active (D 1 (base)	0.455	0.012	0.044	0.244	0.042	0.342	0.034	0.354	0.388	0.730	1.185
2	0.476	0.012	0.022	0.108	0.012	0.154	0.034	0.398	0.432	0.586	1.062
3	0.074	0.044	0.048	0.188	0.062	0.342	0.046	0.840	0.886	1.302	1.376
4	0.009	0.026	0.024	0.108	0.018	0.176	0.040	1.106	1.146	1.322	1.331
5 (tip)	0.000	0.040	0.040	0.128	0.056	0.264	0.060	1.280	1.340	1.604	1.604
Young (E) 1 (base)	0.174	0.102	0.168	0.184	0.022	0.476	0.200	0.170	0.370	0.846	1.020
2	0.185	0.016	0.052	0.184	0.030	0.282	0.018	0.482	0.500	0.782	0.967
3	0.077	0.036	0.046	0.168	0.064	0.314	0.054	0.684	0.738	1.052	1.129
4 (tip)	0.006	0.050	0.048	0.180	0.068	0.346	0.068	1.042	1.110	1.456	1.462
Stem											
Base	0.190	0.068	0.094	0.256	0.226	0.644	0.082	0.654	0.736	1.380	1.570
Middle	0.222	0.032	0.126	0.428	0.186	0.772	0.078	0.780	0.858	1.630	1.852
Apex	0.278	0.074	0.132	0.608	0.134	0.948	0.066	0.826	0.892	1.840	2.118
Roots	0.208	0.020	0.052	0.068	0.008	0.148	0.142	0.998	1.140	1.288	1.496

* Organic plus inorganic nitrogen.

TABLE II

FRACTIONS OF INORGANIC-, SOLUBLE ORGANIC- AND PROTEINACEOUS-NITROGEN IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR., GROWN IN PLUS-IRON (5 MG. PER L.) SOLUTION CULTURES SUPPLIED WITH NITRATE-NITROGEN

PLANT SECTIONS		MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE										
		INORGAN.	SOLUBLE ORGANIC					PROTEINACEOUS			TOTAL	
		NITRATE	PEPTIDE	AMIDE	MONO-AMINO	BASIC	TOTAL	PROTEOSE AND PEPTONE	PROTEIN	TOTAL	ORGANIC	GEN-ERAL*
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves												
Old (B)	1 + 2 (base)	0.326	0.022	0.048	0.108	0.024	0.202	0.232	0.420	0.652	0.854	1.180
	3	0.029	0.050	0.030	0.132	0.048	0.260	0.418	0.614	1.032	1.292	1.321
	4	0.009	0.070	0.038	0.148	0.050	0.306	0.446	0.886	1.332	1.638	1.647
	5 (tip)	0.000	0.104	0.052	0.184	0.078	0.418	0.382	1.164	1.546	1.964	1.964
Mature (C)	1 (base)	0.557	0.030	0.038	0.148	0.026	0.242	0.154	0.120	0.274	0.516	1.073
	2	0.535	0.044	0.034	0.160	0.040	0.278	0.268	0.368	0.636	0.914	1.449
	3	0.075	0.072	0.034	0.156	0.060	0.322	0.454	0.802	1.256	1.578	1.653
	4	0.022	0.082	0.040	0.148	0.074	0.344	0.444	1.000	1.444	1.788	1.810
	5 (tip)	0.010	0.108	0.048	0.192	0.086	0.434	0.474	1.196	1.670	2.104	2.114
Active (D)	1 (base)	0.625	0.062	0.054	0.212	0.038	0.366	0.206	0.192	0.398	0.764	1.389
	2	0.521	0.066	0.072	0.164	0.028	0.330	0.194	0.314	0.508	0.838	1.359
	3	0.079	0.062	0.040	0.220	0.080	0.402	0.362	0.616	0.978	1.380	1.459
	4	0.015	0.112	0.054	0.216	0.158	0.540	0.474	1.220	1.694	2.234	2.249
	5 (tip)	0.000	0.138	0.066	0.284	0.180	0.668	0.474	1.580	2.054	2.722	2.722
Young (E)	1 (base)	0.506	0.136	0.064	0.232	0.048	0.480	0.332	0.386	0.718	1.198	1.704
	2	0.268	0.060	0.038	0.148	0.034	0.280	0.230	0.386	0.616	0.896	1.164
	3	0.044	0.090	0.036	0.164	0.072	0.362	0.328	0.656	0.984	1.346	1.390
	4 (tip)	0.000	0.092	0.046	0.168	0.150	0.456	0.438	1.254	1.692	2.148	2.148
Stem												
Base		0.318	0.158	0.094	0.292	0.092	0.636	0.212	0.574	0.786	1.422	1.740
Middle		0.529	0.270	0.124	0.372	0.116	0.882	0.430	0.506	0.936	1.818	2.347
Apex		0.595	0.360	0.154	0.452	0.120	1.086	0.488	0.582	1.070	2.156	2.751
Roots		0.278	0.026	0.018	0.024	0.018	0.086	0.290	0.286	0.576	0.662	0.940

* Organic plus inorganic nitrogen.

TABLE III

FRACTIONS OF SOLUBLE ORGANIC- AND PROTEINACEOUS-NITROGEN IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR., GROWN IN MINUS-IRON SOLUTION CULTURES SUPPLIED WITH AMMONIUM NITROGEN

Plant sections		Milligrams of nitrogen per gram of fresh tissue								
		Soluble organic					Proteinaceous			Total organ.
		Peptide	Amide	Mono-amino	Basic	Total soluble organ.	Proteose and peptone	Protein	Total protein-aceous	
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves										
Old (B)	1+2 (base)	0.046	0.278	0.324	0.052	0.700	0.056	0.724	0.780	1.480
	3	0.064	0.172	0.476	0.238	0.950	0.074	1.172	1.246	2.196
	4	0.124	0.142	0.480	0.410	1.156	0.100	1.696	1.796	2.952
	5 (tip)	0.120	0.160	0.460	0.536	1.276	0.112	1.936	2.048	3.324
Mature (C)	1 (base)	0.038	0.324	0.376	0.032	0.770	0.054	0.428	0.482	1.252
	2	0.046	0.284	0.428	0.058	0.816	0.046	0.712	0.758	1.574
	3	0.088	0.200	0.472	0.222	0.982	0.074	1.334	1.408	2.390
	4	0.110	0.178	0.520	0.312	1.120	0.100	1.770	1.870	2.990
	5 (tip)	0.124	0.192	0.584	0.388	1.288	0.104	2.238	2.342	3.730
Active (D)	1 (base)	0.044	0.246	0.396	0.038	0.724	0.046	0.368	0.414	1.138
	2	0.036	0.110	0.200	0.040	0.386	0.028	0.550	0.578	0.964
	3	0.100	0.164	0.420	0.122	0.806	0.048	1.126	1.174	1.980
	4	0.140	0.098	0.544	0.406	1.188	0.064	1.664	1.728	2.916
	5 (tip)	0.160	0.206	0.456	0.456	1.278	0.116	2.086	2.202	3.480
Young (E)	1 (base)	0.050	0.140	0.268	0.086	0.544	0.072	0.604	0.676	1.220
	2	0.046	0.124	0.256	0.064	0.490	0.028	0.642	0.670	1.160
	3	0.090	0.152	0.344	0.160	0.746	0.046	1.048	1.094	1.840
	4+5 (tip)	0.110	0.164	0.496	0.374	1.144	0.060	1.778	1.838	2.982
Stem										
Base		0.054	0.214	0.400	0.146	0.814	0.144	0.878	1.022	1.836
Middle		0.062	0.344	0.520	0.152	1.078	0.144	0.728	0.872	1.950
Apex		0.078	0.336	0.556	0.174	1.144	0.176	0.774	0.950	2.094
Roots		0.032	0.092	0.120	0.022	0.266	0.106	0.804	0.910	1.176

tively, whereas those in the combined sections of the leaves and stem were greater in the F than O cultures, being 30.7 $\left(\frac{1.503-1.150}{1.150}\times 100\right)$ and 5.89 $\left(\frac{2.286-2.159}{2.159}\times 100\right)$ per cent. for the N-n and A-n series, respectively.

Nitrate nitrogen

Nitrate nitrogen was more abundant in the F than O cultures of the N-n series as reported in tables I and II. Since both cultures were supplied with equal amounts of nitrate nitrogen, the higher nitrate content of the F cultures resulted from a greater rate of absorption of this ion by these plants, which was confirmed subsequently by certain studies (unpublished) where the nitrate content of nutrient solutions of F and O cultures was determined at the beginning and end of the 2-week period of plant growth. The low nitrate content of the plants of the O cultures in the N-n series resulted from a decreased rate of nitrate absorption which might have been initiated by the depressed rate of growth and metabolic activity on account of the low chlorophyll content of the leaves. The data showing that the weights of the roots were approximately the same in the O and in the F cultures of the N-n series emphasize that the greater content of nitrate in the F cultures was not caused by differences in root surface area but by the differential growth rate and physiological activity as postulated above. The data suggest, moreover, that chlorophyll *per se* did not take direct part in the mechanism of nitrate nitrogen reduction and probably assimilation but that the biochemical and physiological conditions were more propitious for this mechanism in the chlorophyllose than in the non-chlorophyllose regions of the leaves. However, in spite of the low chlorophyll content of the leaves of the O cultures, nitrate nitrogen did not accumulate in the plants, indicating that its reduction and assimilation proceeded satisfactorily.

Ammonium nitrogen

The values of ammonium nitrogen were low in the plants of both series and ranged as mentioned before from 0.002 to 0.020 mg. per gram of fresh tissue. Since inorganic nitrogen from ammonium salts is readily assimilated in the tissues of the roots and of other organs (**31**) the small amounts encountered might have originated in hydrolyzed organic compounds. Recent investigations have shown that this fraction of nitrogen might be derived from glutamic or carbamic acid peptides which form from proteins undergoing hydrolysis, according to Melville (**22**) and Corwin and Damerel (**16**).

Soluble organic nitrogen

The amounts of soluble organic nitrogen, reported in tables I to IV, represent the sum of the amide-, mono-amino-, basic-, and peptide-nitrogen fractions. The proteose-peptone-nitrogen fraction was not included because of its closer relationship to proteinaceous- than to crystalloid-nitrogen.

TABLE IV

FRACTIONS OF SOLUBLE ORGANIC- AND PROTEINACEOUS-NITROGEN IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR., GROWN IN PLUS-IRON SOLUTION CULTURES SUPPLIED WITH AMMONIUM NITROGEN

PLANT SECTIONS	MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE								
	SOLUBLE ORGANIC					PROTEINACEOUS			TOTAL ORGAN.
	PEPTIDE	AMIDE	MONO-AMINO	BASIC	TOTAL SOLUBLE ORGAN.	PROTEOSE AND PEPTONE	PROTEIN	TOTAL PROTEIN-ACEOUS	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves									
Old (B) 1+2 (base)	0.044	0.344	0.360	0.046	0.794	0.110	0.654	0.764	1.558
3	0.058	0.224	0.576	0.128	0.986	0.054	1.462	1.516	2.502
4	0.066	0.290	0.532	0.310	1.198	0.098	2.052	2.150	3.348
5 (tip)	0.090	0.324	0.652	0.430	1.496	0.116	2.264	2.380	3.876
Mature (C) 1 (base)	0.034	0.334	0.300	0.044	0.712	0.118	0.378	0.496	1.208
2	0.050	0.274	0.320	0.092	0.736	0.068	0.864	0.932	1.668
3	0.048	0.166	0.444	0.130	0.788	0.058	1.658	1.716	2.504
4	0.082	0.174	0.484	0.196	0.936	0.092	2.142	2.234	3.170
5 (tip)	0.080	0.170	0.632	0.296	1.178	0.104	2.500	2.604	3.782
Active (D) 1 (base)	0.038	0.268	0.384	0.050	0.740	0.078	0.344	0.422	1.162
2	0.022	0.182	0.184	0.042	0.430	0.026	0.606	0.632	1.062
3	0.050	0.100	0.364	0.122	0.636	0.044	1.446	1.490	2.126
4	0.092	0.138	0.532	0.232	0.994	0.060	2.100	2.160	3.154
5 (tip)	0.092	0.186	0.584	0.350	1.212	0.086	2.476	2.562	3.774
Young (E) 1 (base)	0.098	0.330	0.324	0.070	0.822	0.142	0.330	0.472	1.294
2	0.038	0.084	0.236	0.070	0.428	0.030	0.636	0.666	1.094
3	0.068	0.084	0.368	0.110	0.630	0.034	1.190	1.224	1.854
4+5 (tip)	0.102	0.114	0.540	0.220	0.976	0.076	1.918	1.994	2.970
Stem									
Base	0.064	0.196	0.376	0.064	0.700	0.174	0.874	1.048	1.748
Middle	0.072	0.320	0.444	0.092	0.928	0.188	0.790	0.978	1.906
Apex	0.076	0.410	0.624	0.128	1.238	0.182	0.830	1.012	2.250
Roots	0.028	0.096	0.124	0.016	0.264	0.052	0.620	0.672	0.936

Soluble organic nitrogen was higher in the meristematic tissues, namely in the basal-leaf (no. 1) and apical-stem sections, than in the adjacent transitional (no. 2) sections of the D and E leaves. In the chlorophyllose sections (no. 3, 4, and 5) the values increased to higher levels than in the basal (no. 1) sections. The values of soluble organic nitrogen in the tissues were higher in the A-n than in the N-n series, indicating that more ammonium- than nitrate-nitrogen was absorbed and assimilated by the plants. The effects of the F vs. the O cultures on the soluble organic nitrogen content of the tissues differed in the two series. In the N-n series the values of nitrogen were, with few exceptions, higher in the F than in the O cultures. In the A-n series they were only higher in the meristematic tissues; namely, in the basal (no. 1) sections of the active (D) and young (E) leaves and in the apical section of the stem of the F cultures, but in all other sections except in the old (B) leaves they were higher in the O series.

Amide nitrogen

Amide nitrogen values reported in tables I to IV and depicted in figure 1, were greater in the A-n than in the N-n series.

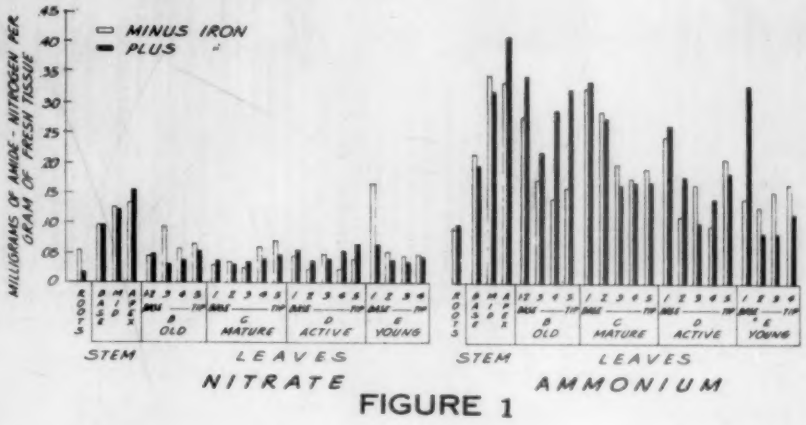

FIGURE 1

Fig. 1. Amide-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

The effects of plus- or minus-iron cultures on the amide nitrogen content of the tissues are confusing. Amide nitrogen values in the basal (no. 1) sections of all groups of leaves, except in the young (E) of the N-n series, were higher for the F than O cultures. The amide nitrogen content of the young (E) leaves, except of the basal section of the A-n series, was higher in the O than F cultures. Uniformly higher values for amide nitrogen occurred in the leaves of the F cultures of the A-n series. In all other groups of leaves amide nitrogen values varied considerably in comparable sections between the F and O cultures.

Amide nitrogen was higher in the apical sections of the stem of the F cultures but lower in the medial and basal sections. This fraction is gen-

erally more abundant in meristematic than in highly differentiated tissues of most plants, and the present findings are in complete agreement with data formerly reported (31) on the distribution of asparagine in *A. comosus*.

Mono-amino-nitrogen

Mono-amino-nitrogen, comprising a great portion of alpha-amino-nitrogen, determined but not reported here, and other fractions than those determinable by the Van Slyke method are reported in tables I to IV and depicted in figure 2. This fraction was equally high in the leaves and stem of the A-n series, whereas in the N-n series the stem contained greater amounts than the leaves.

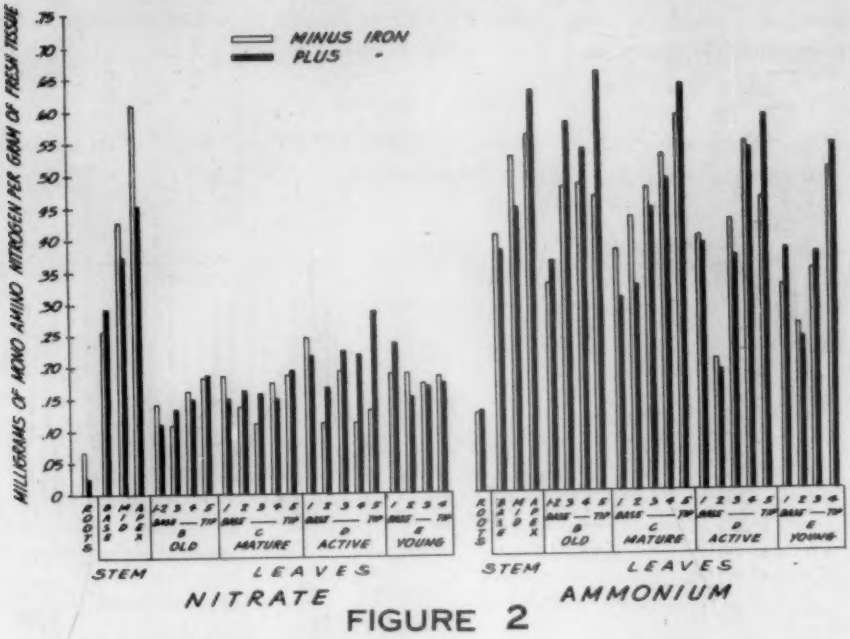

FIGURE 2

FIG. 2. Mono-amino-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

Mono-amino-nitrogen was high in meristematic tissues such as the basal (no. 1) sections of the active (D) and young (E) leaves and the apex of the stem. Its values increased gradually from the low chlorophyllose (no. 3) to the terminal chlorophyllose (no. 5) sections of the leaves in the active (D) and young (E) groups. In the other groups of leaves the gradient of distribution varied slightly.

The effects of plus- or minus-iron cultures on the mono-amino-nitrogen content of plant sections cannot be clearly defined from the data in figure 6. In the young (E) leaves the basal (no. 1) sections of the F cultures contained higher amounts of mono-amino-nitrogen in both series. In the other sections of these leaves, except the transitional (no. 2), the amounts for the F cul-

tures were high in the A-n series but low in the N-n series. In the active (D) leaves mono-amino-nitrogen was higher in all sections, except in the terminal (no. 5), of the O than F cultures in the A-n series. In the N-n series these values were higher for the F than O cultures except in the basal (no. 1) section. In the mature (C) leaves the values were high for the F cultures in the A-n series but not in the N-n series as shown in figure 6. More mono-amino-nitrogen accumulated in all sections of the stem in the O than in the F cultures except in the apical of the A-n and basal of the N-n series.

Basic nitrogen

Basic nitrogen, containing some alpha-amino-nitrogen and certain other not well defined fractions, is reported in tables I to IV and depicted in figure 3. The amounts of this faction were higher in the chlorophyllose sections of the leaves of the A-n than in the N-n series. The differences in the amounts of basic nitrogen in the (no. 1) basal non-chlorophyllose sections of the leaves and in the stem between the A-n and N-n series were small. Basic nitrogen values were generally higher in the O than in the F cultures of the A-n series. However, a few insignificant exceptions were noted. In the N-n series, these values were greater in most leaf sections of the F than O cultures, exceptions being the basal (no. 1) sections of the active (D), mature (C), and old (B) leaves, and the medial chlorophyllose (no. 4) section of the latter group. In the stem these values were reversed, being higher in the O than in the F cultures.

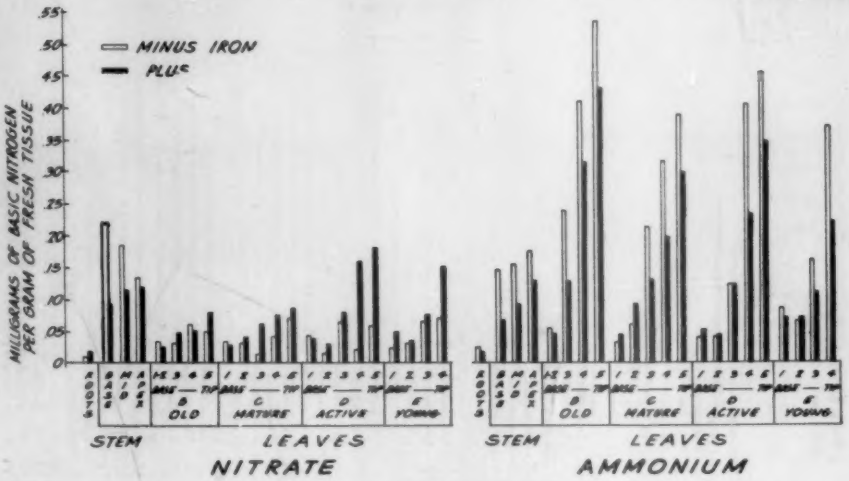

FIGURE 3

Fig. 3. Basic-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

Peptide nitrogen

The amounts of peptide nitrogen, reported in tables I to IV and depicted in figure 4, differed considerably between the F and O cultures of the N-n

series, whereas in the A-n series such differences were small. The differences in the N-n series were from 2 to 8 times greater in the F than O cultures and were statistically significant, as reported in table V. Peptide nitrogen in the A-n series was relatively more abundant in the leaves of the O than of the F cultures but such differences were not highly significant statistically. Fluctuations in the amounts of peptide nitrogen between different plant sections were less pronounced in the F than in the O cultures for both nitrogen series.

Proteose and peptone nitrogen

These combined fractions of nitrogen, although soluble in the extracting medium, are related more to the proteinaceous- than the crystalloid-nitrogen fractions.

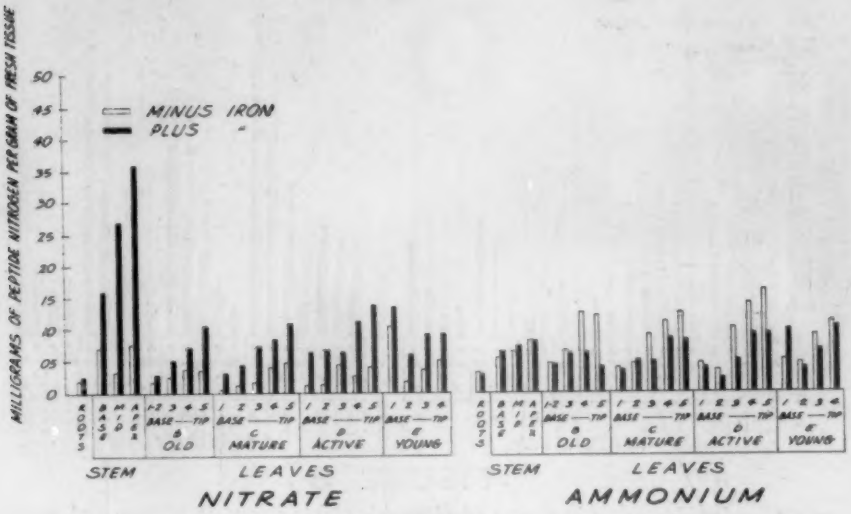

FIGURE 4

Fig. 4. Peptide-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

The amounts of the combined proteose-peptone nitrogen fraction, reported in tables I to IV and depicted in figure 5, were greater in the F than in the O cultures of the N-n series. The difference of the means was statistically significant, as reported in table V. Such differences between the F and O cultures in the A-n series were small and inconsistent and lacked statistical significance. However, in the meristematic tissue of the leaves, namely in the basal (no. 1) section, proteose-peptone nitrogen values were higher in the F cultures, whereas in most other sections (3, 4, and 5) they were higher in the O cultures.

The proteose-peptone nitrogen content of the stem was higher in the F cultures of both series.

Protein Nitrogen

The amounts of protein nitrogen of the plant tissues, reported in tables I to IV and depicted in figure 6, were affected by the sources of inorganic nitrogen and the amounts of available iron in the nutrient solution. Such amounts were also influenced by the physiological status and the functional specialization of the tissues in the different sections. For example, the plants in the A-n series contained more protein nitrogen than in the N-n series because the rate of ammonium nitrogen absorption from the solution culture and its subsequent assimilation by the tissues was greater than that of nitrate nitrogen, as reported in a former study (**31**). Also, the amounts of protein nitrogen in the chlorophyllose sections of the A-n and in many of the N-n series of the leaves were greater for the F than for the O cultures.

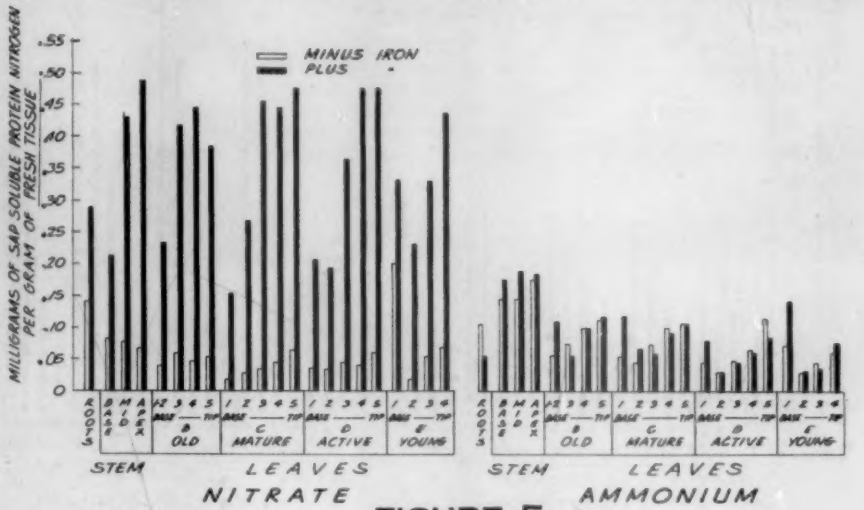

FIGURE 5

FIG. 5. Sap-soluble, inorganic-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

Chlorophyll differences between the F and O cultures in the A-n were small as compared to those between the same cultures in the N-n series. However, the differences in amounts of protein nitrogen of comparable sections between the F and O cultures were greater in the A-n than in the N-n series. These comparisons reveal that certain other factors in addition to the chlorophyll content of the leaves affected the protein content of the tissues. Protein nitrogen values in the stem, being greater for the O than F cultures in the N-n series, suggest that they resulted from concentration effects on account of smaller stem weights in the former (428 gm.) than in the latter cultures (609 gm.) However, the protein nitrogen content of the stems in the A-n series, where stem weights for both cultures were approximately the same, was greater in the F than in the O cultures and behaved in this respect like the leaves.

Discussion

The data on the effects of iron in the assimilation and in the subsequent metabolic changes of ammonium- or nitrate-nitrogen by *A. comosus* suggest that iron had affected the course of such processes only indirectly. The direct effects of iron can be traced only as far as the formation of chlorophyll. It appears that beyond this point chlorophyll more than iron dominated the course of the various physiological processes. Data presented in the second paper of this series (**31**) showed that the plants of the O cultures of the N-n series contained smaller quantities of chlorophyll than any of the other cultures. The same plants also contained, with the exception of a few

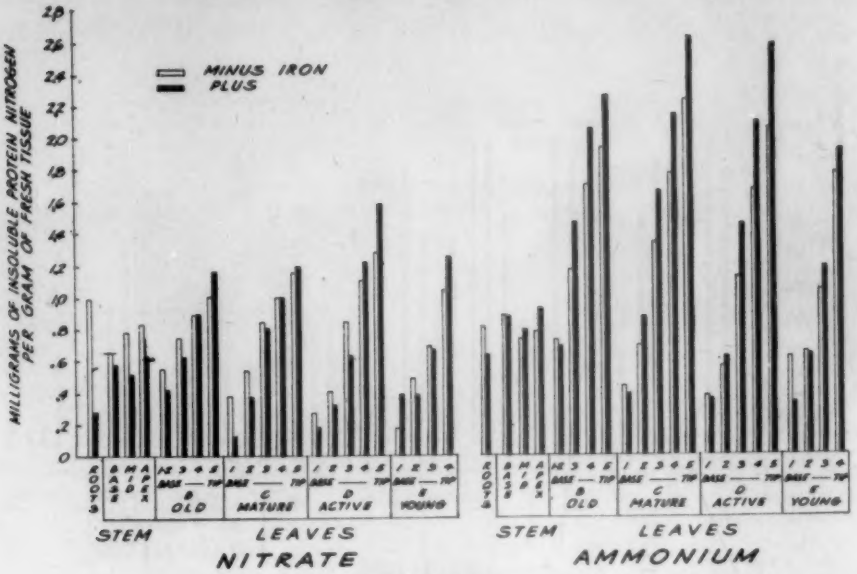

FIGURE 6

FIG. 6. Insoluble-protein-nitrogen in tissues of pineapple plants grown in nitrate- and ammonium-nitrogen cultures.

sections, smaller amounts of total organic-, nitrate-, soluble organic-, peptide-, and proteose-nitrogen. Differences between the O and F cultures of the N-n series in mono-amino-, basic-, amide-, and protein-nitrogen fractions were not consistent. The greater amounts of total organic- and nitrate-nitrogen in the plants of the F than O cultures of the N-n series should be attributed to a higher rate of absorption and assimilation of nitrate nitrogen by the former plants. The data also suggest that optimum amounts of chlorophyll coupled with favorable conditions for uninterrupted carbohydrate synthesis were essential factors for a high rate of nitrate absorption and assimilation.

The amounts of the various nitrogenous fractions varied between comparable sections of the plants of the F and O cultures in the two series. Also,

the amounts of certain nitrogen fractions differed appreciably in the two series. For instance, the amounts of mono-amino-, basic-, amide-, and protein-nitrogen were greater in the A-n series, whereas those of peptide- and proteose-peptone-nitrogen were greater in the N-n series.

In many leaf and stem sections of the A-n series the amounts of soluble organic nitrogen were greater in the O than in the F cultures, which might indicate that either certain fractions in the soluble organic nitrogen were not converted into proteins as rapidly in the O as in the F cultures because of restricted plant growth or that proteins were hydrolyzed at a greater rate in the O than F cultures.

Examination of the data in the light of recent investigations on nitrogen metabolism suggest a somewhat different course for the assimilation of

TABLE V

CALCULATED STATISTICAL SIGNIFICANCE, BY CONRAD'S FORMULA AND FROM LOVE'S TABLE, OF THE DIFFERENCE BETWEEN THE AMOUNTS OF THE SUBSTANCES LISTED BELOW OF COMPARABLE SECTIONS OF THE LEAVES AND STEM OF PLANTS GROWN EITHER IN "PLUS IRON" OR IN "MINUS IRON" CULTURES AND SUPPLIED EITHER WITH NITRATE OR AMMONIUM SALTS AS SOURCES FOR NITROGEN

Substances	Nitrate-N				Ammonium-N			
	Leaves		Stem		Leaves		Stem	
	Significance	In favor of culture	Significance	In favor of culture	Significance	In favor of culture	Significance	In favor of culture
Organic-N	9999 : 1	+ Fe	55 : 1	+ Fe	5000 : 1	+ Fe	None	.....
Peptide-N	9999 : 1	+ Fe	25 : 1	+ Fe	30 : 1	− Fe	None	.....
Amide-N	None	.....	None	.....	None	.....	None	.....
Mono-amino-N	None	.....	None	.....	22 : 1	+ Fe	None	.....
Basic-N	100 : 1	+ Fe	None	.....	3000 : 1	− Fe	65 : 1	− Fe
Proteose-N	9999 : 1	+ Fe	25 : 1	+ Fe	None	.....	None	.....
Protein-N	None	.....	25 : 1	− Fe	9999 : 1	+ Fe	None	.....

ammonium and nitrate nitrogen. SIDERIS, KRAUSS, and YOUNG (**30, 31**) have shown in *Pandanus veitchii* Hort., and in *Ananas comosus* (L.) Merr., and VICKERY *et al.* (**36**) in *Nicotiana tabacum* L., that the amounts of amide-N and amino-N were greater when these plants were supplied with ammonium nitrogen but smaller when the source of nitrogen was nitrate. The above mentioned results indicated that the primary products of ammonium assimilation were amide-N and amino-N compounds and suggest that protein synthesis in plants directly absorbing ammonium may be via these primary products. But in the plants absorbing nitrate from solution cultures the small amounts of amide-N and amino-N compounds suggest a slightly different course for the assimilation of nitrate than that of ammonium. BARTON-WRIGHT and MCBAIN (**3**) claim that in *Solanum tuberosum* L. protein synthesis is direct from nitrate-N via resident-N and probably not via amino acids. The residual-N fraction reported by these authors is comparable to the combined peptide-proteose-peptone fraction reported in these studies.

The presence of proteose-nitrogen in plants has interested many investigators. McKie (21) claims that, "proteoses are the precursors and immediate decomposition products of the proteins and also that, while proteins are being rapidly synthesized in plants, proteoses diminish and vice versa." That proteins are susceptible to splitting by plant enzymes into disaggregates comparable to proteoses has been shown by Blagoveshchenski and Sossiedov (10) and Blagoveshchenski and Yurgenson (11) who claim that flour enzymes disaggregate proteins without setting free amino groups from polypeptide chains, indicating that the peptide linkage is not being attacked.

Recent investigations have thrown some light on the mechanism of biological synthesis of amino acids, polypeptides, and proteins in plant and animal tissues. Studies by Braunstein and Kritzman (12, 13) and Cohen (14) have shown that in plant and animal tissues ammonia is transferred by transaminases, a group of enzymes, from an amino acid to a ketonic acid, with the amino acid being oxidatively deaminated and the ketonic acid reductively aminated. The transfer of ammonia to mono- or di-carboxylic ketonic acids (e.g., pyruvic or oxaloacetic) is directly from dicarboxylic amino acids (glutamic or aspartic) but not from mono-carboxylic amino acids. Certain monocarboxylic acids (lysine, phenylalanine, serine, and leucine) may act as secondary NH_3 donators, but direct transference of NH_3 to dicarboxylic ketonic acids is not possible except through the presence of catalytic amounts of dicarboxylic amino acids.

Amines and peptides cannot function as NH_3 donors. The studies of Adler *et al.* (1), Albaum and Cohen (2), and Vitanen *et al.* (37, 38) are in support of the theory of transamination as postulated above. Other investigations have elucidated certain phases in the mechanism of peptide synthesis in biological systems. Studies *in vitro* by Herbst and Sherwin (18) in which dl-alanylalanine was synthesized by transamination from pyruvylalanine and a-aminophenylacetic acid as the amino donor suggest a scheme for the biological synthesis of peptide chains from non-amino precursors involving amination and acylation. Bergmann and his colleagues (5, 6) have provided evidence in the synthesis and hydrolysis, by proteinases, of simple peptides from amino acids in the presence of an activator such as cystine. These investigators (4, 8, 9, 10) showed that papain, chymotrypsin, and bromelin were capable of synthesizing amide bonds, such as the synthesis of benzoyl-l-phenylalanyl-l-leucine anilide from benzoyl-l-phenylalanine and l-leucine anilide, indicating recombination of some of the amino acid residues by the synthesis of new peptide bonds in addition to the scission of peptide linkages from proteins. Bromelin is distributed in all organs of *A. comosus* (28), and synthesis of peptide bonds or the scission of peptide linkages from proteins under the conditions of nitrate or ammonium nutrition may affect the peptide and proteose content of the tissues.

High concentrations of amino nitrogen, as shown in the plants of the ammonium series, have been variously explained by different investigators.

PETRIE (23) and PETRIE and WOOD (24) claim that, "a given relation of proteins to amino acids will hold only so long as the proportions between the individual amino acids remain constant; the relation will be different for every change in amino acid proportions." They have based their statement on data which showed that cystine increased less rapidly than other amino acids when the total concentration increased. LUGG and WELLER (19) have also reported a comparable condition where protein synthesis was limited by the low amounts of methionine in the seed.

In this study, the higher concentrations of amide-, amino-, and protein-nitrogen in the ammonium than in the nitrate series may be explained by the greater reactivity of ammonium with dicarboxylic ketonic acids, assuming that these substances are present in the tissues, to form either glutamic or aspartic acids or their corresponding amide fractions as well as other amino acids. The biochemical conditions which are required for the reduction of nitrate and its conversion to organic compounds which, according to our information to date, takes place in the chlorophyllose tissues of the leaves, may retard the rate of synthesis of amide- or amino-nitrogen fractions and thus explain the low amounts of amide- or amino-nitrogen fractions. The physiological mechanism for the reduction of nitrates in the tissues of *A. comosus* is unknown. However, one or more activators which cause the conversion of nitrates into some organic nitrogen fractions, comparable to cystine in the studies of BERGMANN and his colleagues, may be present in the chlorophyllose and lacking in the non-chlorophyllose tissues of the leaves. Such fractions if identical with the amide- and amino-nitrogen in the plants of the ammonium series must have polymerized immediately to such complex compounds as polypeptides.

STEWART and PRESTON (34) have stressed oxygen tension as playing an important rôle in protein synthesis and salt accumulation on the basis of studies on the aeration vs. non-aeration of potato discs suspended in nutrient solutions. In our study the plants of both ammonium and nitrate series were aerated equally. However, molecular oxygen released from reduced NO_3 molecules might have increased the intracellular tension of oxygen which may act as a catalyst in the polymerization of amide- or amino-nitrogen to polypeptides and proteins.

The data definitely indicate that high amounts of amide- and amino-nitrogen in tissues of *A. comosus* were associated with a source of ammonium in the solution culture while nitrate-, peptide-, or proteose-peptone-nitrogen with one of nitrates. However, different amounts of other nutrient elements or conditions of light and temperature may change materially the amounts of such nitrogen fractions in the tissues by their effects on the carbohydrate reserves and rate of mineral absorption which in turn influence the nitrogen assimilating capacity of plants.

Summary

1. The plants grown in the ammonium series contained greater amounts of total nitrogen than those in the nitrate nitrogen series. The leaves and

stem but not the roots of the plants of the plus-iron cultures in the ammonium-nitrogen series contained slightly greater amounts of total nitrogen than of the minus-iron cultures. The differences in total nitrogen between the plus- and minus-iron cultures were greater in the nitrate- than ammonium-nitrogen series, and they were in favor of the former cultures.

2. The amide- and amino-nitrogen content of the tissues was greater in the ammonium- than in the nitrate-nitrogen series. In the former series the plus-iron cultures contained more amide- and amino-nitrogen than the minus-iron cultures. In the nitrate-nitrogen series, the plus-iron cultures contained more peptide- and proteose-peptone-nitrogen than the minus-iron cultures. These results indicate that the primary products of ammonium assimilation were amide and amino nitrogen compounds and suggest that protein synthesis in plants supplied with ammonium nitrogen was via these compounds. In the plants supplied with nitrate, protein synthesis from inorganic nitrogen compounds was apparently different than with ammonium.

3. Statistically significant differences were obtained in the amounts of organic-N, peptide-N, basic-N and proteose-peptone-N between the plus- and minus-iron cultures of the nitrate-nitrogen series which were in favor of the plus-iron cultures, whereas in the ammonium-nitrogen series such differences were obtained in the organic-N and protein-N fractions. In the ammonium-nitrogen series, basic nitrogen was the only fraction highly significant in favor of the minus-iron cultures.

UNIVERSITY OF HAWAII
HONOLULU, T. H.

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TRANSPIRATION OF PINE SEEDLINGS AS INFLUENCED BY FOLIAGE COATINGS

HUBERT MARSHALL AND T. E. MAKI

(WITH ONE FIGURE)

Widespread losses frequently occur in conifer plantations during the first weeks after the young trees are set in the field. This early mortality is largely attributable to "physiological drought" resulting from continued transpiration and the concomitant failure of the recently disturbed root systems to make up the daily water deficit. Under such conditions permanent wilting occurs, and death soon follows.

In attempting to reduce this mortality, methods have been sought to encourage the early establishment of the root system and to reduce the excess of transpiration over absorption during the establishment period. This latter approach to the problem has been used with gratifying success by nurserymen who frequently top-prune hardwoods as a means of reducing transpiration and of increasing survival of transplanted stock. Reducing the transpiring surface of conifers by this method has not proved feasible, but it is possible that coating the foliage with various waxes, fats, and oils might produce an equally effective reduction in water loss. This has been attempted but with variable results both in terms of water loss and field survival; to date the practice has not received widespread acceptance.

The literature contains but slight mention of the degree to which transpiration of coniferous seedlings is reduced by foliage coatings; unfortunately the information available has generally been obtained by the use of coatings which, under some conditions at least, have proved of little value for increasing resistance to drought. EMERSON and HILDRETH (**1**), for example, found that corn oil and several other substances reduced water loss from dormant *Pinus austriaca* seedlings as much as 88.4 per cent. and caused no foliage injury under conditions prevailing in Wyoming. No actual test of drought resistance was reported. However, in tests at Beltsville, Maryland (**2**), under both field and artificial drought conditions, corn oil caused injury to the foliage of a number of coniferous species and did not materially increase resistance to drought. In similar tests, SHIRLEY and MEULI (**4**) studied the effects of 1 rubber and 3 wax coatings on two-year-old seedlings of white spruce. Two of the coatings materially reduced transpiration, but in extensive field trials with several species none of the substances increased survival.

A number of experiments (**2**) conducted during the past several years, both in the field and under conditions of artificial drought, have shown that foliage coatings of emulsified lanolin and a commercial paraffin wax increase first year survival about 10 per cent. Under more specific conditions, such as late fall planting, foliage coatings of these substances may produce even greater benefits. The purpose of this report is to present data showing the

reductions in transpiration resulting from the use of these two coatings under conditions of atmospheric drought. Incidental observations on the relation of transpiration to root volume for several species also are presented.

Materials and methods

The species used in this study were 2–0 loblolly pine (*Pinus taeda*), 5–0 red pine (*Pinus resinosa*),[1] and 3–0 white pine (*Pinus strobus*), lifted from a local nursery in July, 1944. Thirty uniform seedlings of each species were selected, weighed individually, and divided into three groups of roughly equal weight for treatment with foliage coatings. The following three treatments were used:

1. Control (untreated).
2. Lanolin emulsion at a concentration of 100 grams lanolin (anhydrous Adeps Lanae) per liter of water.
3. Dowax,[2] one part prepared emulsion to three parts water.

The lanolin was emulsified with 10 grams of monoethanolamine stearate per liter of water. The commercial wax required only mixing with three times its weight of water. The seedlings were treated by immersion of the foliage in the proper substance for a few moments followed by a gentle shaking to remove excess coating material.

After treatment the seedlings were potted in no. 2 tin cans measuring 2½ inches in diameter and 3½ inches in depth. The potting medium was a fine sand mixed in bulk to a moisture content of 18.2 per cent. based on dry weight. This moisture content was at the field capacity of the sand and was only slightly reduced during the course of the experiment. Water loss was determined daily by weighing the cans on a small trip balance sensitive to 0.1 gram. To prevent loss of water by evaporation from the surface of the sand, the cans were effectively sealed with a half and half mixture of paraffin and beeswax.

During the course of the experiment, the seedlings were exposed to the environmental conditions of the drought machine described by SHIRLEY and MEULI (5). Briefly, this machine consists of a revolving circular table six feet in diameter, enclosed in an insulated metal drum four feet in height. The seedlings rest on the revolving table and are exposed to a thermostatically controlled temperature of 96° F., constant light intensity of about 200 foot-candles from two incandescent bulbs, and a wind velocity of several miles per hour generated by two fans attached to the inner sides of the metal drum. The revolving table is driven by an electric motor and has a velocity at the periphery of 1.4 miles per hour. Humidity in the present test was approximately 38 per cent.

[1] The 5–0 red pine, while not ordinarily used in forest plantings, was the only size class available and provided a satisfactory test object for the purposes of this study.

[2] The commercial wax used in this study is the proprietary product, Dowax, containing a paraffin wax, bentonite, an emulsifying agent, and possibly other substances. The exact composition is unknown to the writers.

At the conclusion of the 5-day exposure period, root volumes of all seedlings were measured to the nearest 0.1 ml. by water displacement in a burette. This information was obtained in order to correlate root volume with transpiration.

Results and discussion

Four-day water loss

Despite the existence of ample moisture in the sand, several control seedlings showed some slight evidence of drought stress at the end of the fourth day of exposure. This "physiological drought" is presumably similar to that of seedlings showing drought stress shortly after planting in moist soils in the field. Since the data collected beyond this time were not representative of vigorous seedlings, the results for the first four days are chosen as representing the effects of foliage coatings in the period immediately after planting. The data are shown in table I.

TABLE I

Water loss from groups of 10 seedlings exposed for 4 days to artificial atmospheric drought

Species	Treatment	Mean fresh weight of seedlings	Water loss in 4 days		
			Actual mean	In relation to fresh weight	In relation to controls
		gm.	*gm.*	%	%
White pine	Control	12.33	27.30	221.41	100.00
	Lanolin	12.60	10.52	83.49	37.71
	Com'l. wax	12.75	10.03	78.67	35.53
Red pine	Control	10.83	16.45	151.89	100.00
	Lanolin	11.01	7.10	64.49	42.46
	Com'l. wax	11.39	5.41	47.50	31.27
Loblolly pine	Control	5.77	9.33	161.70	100.00
	Lanolin	6.01	4.64	77.20	47.74
	Com'l. wax	5.85	3.39	57.95	35.84

Differences in fresh weight within species were not found to be significant when tested by Snedecor's analysis of variance method (**6**). The column showing water loss as percentage of fresh weight provides a comparison of treatment differences in which water losses for all three species are established on the same relative basis. The final column provides the better comparison between controls and the two foliage coatings and shows that treated plants on the average transpired less than 40 per cent. as much as controls. Such a reduction in transpiration presumably is responsible for the increased resistance to drought shown by treated seedlings planted under many conditions in the field.

The differences between control and treated seedlings (table I) were tested by the analysis of variance method and were found to be highly significant. The differences between seedlings treated with lanolin and com-

mercial wax likewise were tested, but highly significant differences were found for only red pine and loblolly pine. Although this latter test gives an estimate of the differences necessary for significance, it should not imply the superiority of any particular coating, since concentrations were arbi-

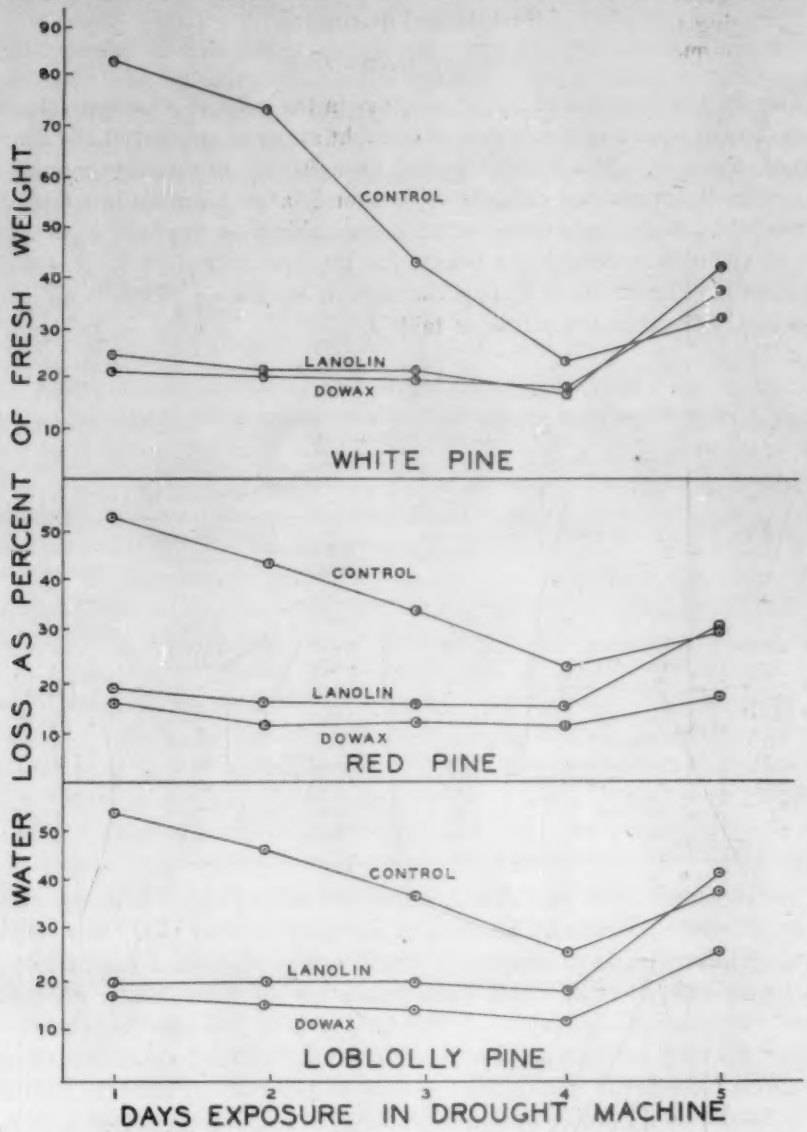

FIG. 1. Water loss of groups of 10 pine seedlings exposed to artificial drought.

trarily selected, and the results would be expected to vary with changes in concentration.

The first four days of exposure provided the basis for the results presented above. On the fifth and final day of exposure, an extra set of bulbs was accidentally illuminated, resulting in a temperature rise from 96° to

110° F. and a drop in the relative humidity from 38 to 21 per cent. At the end of the fifth day all white-pine controls, eight of the ten red-pine controls, and three of the ten loblolly-pine controls showed severe drought injury, and it was apparent that most of the injured seedlings were beyond the point of recovery. Among coated seedlings only three scattered individuals showed a comparable degree of injury, thus providing an indication of the degree of protection afforded by the coatings under drought machine conditions. Samples of sand taken from the cans at the termination of the exposure period showed that all seedlings still had an adequate supply of moisture.

The course of transpiration during the entire 5-day period of exposure is shown graphically in figure 1. Although not proved experimentally, figure 1 indicates that most coated seedlings absorbed sufficient moisture to maintain a uniform daily water loss as modified by the coatings and, either by absorption or the loss of moisture reserves, responded to the drastic conditions of the fifth day of exposure with materially increased transpiration. Control plants, on the other hand, with an initial rate of water loss some three times greater than that of coated plants, were unable to maintain that rate for even a full day. Despite adequate moisture in the sand, the transpiration rate continued to fall, indicating, presumably, a progressive reduction in both moisture reserves and the rate of water absorption. The rise in water loss on the fifth day was not as large as that for coated seedlings, despite the fact that the original rate of water loss was far greater. Thus, the high rate of transpiration resulting from the absence of foliage coatings so exceeded the rate of absorption that most control seedlings were injured beyond the point of recovery by the end of the fifth day of exposure.

Water loss in relation to seedling size

The data in table I are based on mean values, hence they do not provide information on water loss as related to variations in seedling size within each of the three species. To gain information on this relationship, regression equations of the type formula $Y = a + bX$ were calculated in which Y represents water loss in grams for the four-day period, X represents fresh weight of seedlings in grams, and a represents the Y-intercept.

The equations are as follows:

Control series.	
White pine (Range, 7–19 gm.)	$Y = 0.523X + 20.85$
Red pine (Range, 6–18 gm.)	$Y = 0.957X + 6.09$*
Loblolly pine (Range, 4–11 gm.)	$Y = 0.326X + 7.45$
Lanolin series.	
White pine (Range, 6–19 gm.)	$Y = 0.734X + 1.27$**
Red pine (Range, 6–15 gm.)	$Y = 0.939X - 3.24$**
Loblolly pine (Range, 4–9 gm.)	$Y = 0.893X - 0.73$*
Commercial wax series.	
White pine (Range, 7–20 gm.)	$Y = 0.884X - 1.24$**
Red pine (Range, 6–18 gm.)	$Y = 0.398X + 0.88$**
Loblolly pine (Range, 3–11 gm.)	$Y = 0.525X + 0.32$**

The regression coefficients of the equations marked with single or double asterisks are significant at the 0.05 or 0.01 levels, respectively. Although each equation is based on data from only ten seedlings, more than half of the coefficients are highly significant, thereby reflecting the close association of water loss to seedling weight, a relationship which was demonstrated to be linear within the ranges of the present data. In this regard, it is of interest to note that the commercial wax, and to a lesser extent lanolin, reduced random variation in water loss between individual seedlings sufficiently to afford a statistical verification of the relationship in every instance of the treated series.

Species and root-volume relationships

The data in table I provide an example of the sizable differences in water loss which occur between closely related species growing in the same environment. Little is known regarding the basis for such differences, but it is possible they are associated with morphological factors such as root volume. To test this possibility, root volumes were determined and are compared with water loss in table II.

TABLE II

Water loss* during 4-day drought exposure and root volumes† of red and loblolly pines expressed in terms of those of white pine

Species	Relative water loss by treatments				Relative root volumes by treatments			
	Control	Lanolin	Com'l. wax	Species mean	Control	Lanolin	Com'l. wax	Species mean
	%	%	%	%	%	%	%	%
White pine	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Red pine	68.6	77.2	60.4	68.7	81.4	66.8	58.5	68.9
Loblolly pine	73.0	92.5	73.7	79.7	93.1	80.5	80.1	84.6

* Calculated from water loss per gram fresh weight.
† Calculated from root volume in milliliters per gram fresh weight.

Inspection of the table reveals a similarity between the four-day water loss and root volume. White pine, with the largest water loss, likewise had the largest root volume per unit fresh weight; red pine, with the smallest relative water loss, had the smallest root volume. Not only are these species differences consistent for all three treatments, but the mean values are remarkably similar. Although not proved experimentally, the possibility is suggested that, under the conditions of this experiment, transpiration may be largely a function either of root volume or of some associated factor.

These observations were largely a side issue to the main purpose of the experiment and verification of the water-loss–root-volume relationship under other conditions and with other species has not been made. It is understood that there may not be a close association between root volume and transpiration when species having unlike root systems are compared. Between species having similar root systems, however, the relationship appears valid inasmuch as volume, under these circumstances, is closely correlated with

surface area. The present observations, for example, are of importance in studies such as that of SCHOPMEYER (3), where an attempt was made to relate the drought resistance of several species of conifers to their rates of transpiration. Similar studies might profitably consider the relation of transpiration to root volume before conclusions on the relation of transpiration to drought resistance are formulated.

Summary

The water loss of white pine, red pine, and loblolly pine seedlings was determined gravimetrically during exposure to environmental conditions of high temperature and low humidity. With ample moisture in the potting medium, the following results were obtained:

1. Seedlings top-dipped in emulsions of lanolin or a commercial paraffin wax transpired during four days of exposure less than 40 per cent. as much as untreated seedlings.

2. Untreated seedlings failed to respond to a substantial rise in temperature on the fifth day of exposure with as large an increase in water loss as did coated seedlings. Although not demonstrated experimentally, this response is presumed to result from a severe moisture deficit created in untreated seedlings during the first four days of exposure.

3. On the basis of water loss per unit fresh weight, red and loblolly pines transpired respectively only 68.7 and 79.7 per cent. as much as white pine. The calculation of root volumes per unit fresh weight showed a closely similar relationship.

4. Regression equations demonstrated that water loss varies directly as the fresh weight of the seedling within the range of the present data.

NORTHEASTERN FOREST EXPERIMENT STATION
U. S. FOREST SERVICE
PHILADELPHIA, PENNSYLVANIA

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AN ELECTROMETRIC METHOD FOR DEFINING THE AREA OF BARK AFFECTED BY TAPPING *HEVEA BRASILIENSIS*

DAVID G. WHITE

(WITH TWO FIGURES)

The principal source of natural rubber is the Para rubber tree [*Hevea brasiliensis* (H.B.K.) Muell. Arg.]. Even after several decades of plantation practice no satisfactory method has been developed for measuring the area of bark affected by tapping. Such measurements are important because modern tapping systems sometimes utilize two cuts on the same tree, and the cuts frequently interfere reciprocally (**5, 6, 7, 12**). Thus, the separation of panels by an arbitrary distance often results in yields which do not meet expectations, and, in many instances, a physiological disorder called "Brown Bast" develops. This diseased condition is associated with over-tapping (**10, 11**) and may be caused by the two tapping cuts being too near each other. In parts of some plantations over 80 per cent. of the trees are out-of-tapping because of Brown Bast. Incidence of this magnitude could be avoided if the area of bark influenced by tapping were reasonably defined, and the two cuts spaced accordingly. The purpose of this paper is to explain a method which fulfills the requisites of such measurements. However, the limited number of trees available for the experiment described herein restricts further study at this station. Field applications of this electrometric method would need to be based upon results with select clonal trees found on modern plantations.

Previous methods of measurement

The problem is of sufficient importance that numerous attempts have been made to devise a method to measure the area of bark affected by tapping. Briefly, some of these methods have been: The use of interrupting cuts of various lengths and distances below the tapping cut in such a way that the yield is decreased by cuts made within the border of the "drained" area (**2**); the tapping of trees which produced a colored or oxidizable latex in a known area of bark below the tapping cut and white latex in the area near the cut (**14**); mathematical estimations of the hypothetical area of bark concerned for the daily regeneration of latex during a rest period (**1**); and calculations based upon the volume of latex expressed from a known area of removed bark (**4**). Each method has had at least one key fallacy, such as measuring only the hypothetical area of bark through which latex moved before it exuded. Since the vessels are never empty there is, of course, movement of latex in an area of bark greater than that merely "drained." According to these methods latex moves to the tapping cut over a distance ranging from 1 to 3.5 meters. The consensus of opinion has been that a distance of

1 meter is sufficient separation for two cuts. However, this arbitrary distance does not allow for differences between high- and low-yielding trees. As a result, high-yielding trees have suffered the most from having cuts too near each other.

An electrometric method

The diffusion pressure gradient in the bark below a cut is known to be radically affected by tapping (**2**, **4**, **14**). It appears logical that measurement of the distance below the tapping cut over which this gradient occurs

FIG. 1. The Bouyoucos Bridge used in determining variations in resistance of the bark tissues caused by tapping *Hevea brasiliensis*. Hypodermic needles were employed as terminals inserted in the bark (arrows). The usual type of tapping knife is also shown.

also would define the area of bark influenced by tapping. The measurement of the variations in diffusion pressures of the latex and other cell contents can be performed electrically. A method used in this study was to measure the resistance to an electric current offered by the bark tissues and cell contents, including the latex, at intervals below the tapping cut.

Satisfactory terminals were made from discarded hypodermic needles which were shortened to within $\frac{1}{2}$ inch of the shoulders. The ends were

squared and sharpened. The needles were then inserted firmly in the bark at right angles until woody tissues were encountered. Two terminals were placed exactly 1 inch apart horizontally, and pairs were spaced 6 inches apart vertically (fig. 1). Resistance of the bark tissues to a relatively small electric current was measured in ohms by means of the Bouyoucos Bridge (**3**). Six-foot, radio-wire leads with spring clamps attached on the terminals permitted rapid operation.

Several virgin seedling trees of *Hevea brasiliensis* 20 to 25 years of age were available for experimentation. Unfortunately none of these trees are equal to high-yielding clonal selections, but, nevertheless, the results are applicable insofar as the method is concerned. After preliminary tests with a few trees, the best one was chosen for demonstrating the method. A half-circumference cut was marked to start 50 inches from the ground. Pairs of terminals were inserted beneath the center of the proposed tapping cut and also on the opposite side at the same heights. Several days elapsed before measurements were started so that wound effects, if any, might become constant. However, later experience indicated that the resistance between terminals became relatively constant within a few hours after the insertion of the terminals.

The first electrometric measurements were made without having tapped the tree in order to determine the usual fluctuations which might be expected to occur. These preliminary tests, made while the trees were in leaf, demonstrated diurnal variations in resistances of the bark tissues to an electric current. These variations often coincided with the usual daily transpiration curves of leafy plants (**8, 9**). Further investigations with tomato plants indicated the possibility of refining this method as an indirect measurement of transpiration. However, in the present study, differences caused by transpiration of the rubber tree were negligible in comparison with those caused by tapping.

Results

Electrometric measurements were made at intervals throughout the day for 2 days on the tree shown in figure 1 before the tapping cut was opened. The daily variation in resistance from the initial resistance offered by the bark between individual pairs of terminals was considered an important criterion. In other words, differences between sets of terminals were of no importance as long as they were within a reasonable range of several thousand ohms at the beginning. These beginning differences among sets of terminals located at 6-inch intervals were probably caused by variations in bark structure, the depth to which the terminals penetrated, and the amount of bark separating individual pairs. In any case the electric current followed the course of least resistance between terminals.

The resistances between terminals both beneath the proposed tapping cut and on the opposite side of the trunk varied throughout the day as much as 3,000 to 4,000 ohms. In all instances of daily variation before the tapping cut had been opened there was a decrease in resistance and in no case an

increase. This variation throughout the day is partially explainable on the basis of differences in diffusion pressures caused by translocation (**13**). *Hevea brasiliensis* is deciduous, and the tree had just begun to form new leaves. However, the precise cause (or causes) of the daily fluctuations was not studied.

Radical changes in resistance brought about by tapping, as shown later, occurred within the first half hour after tapping. Therefore, the variation in resistances which might occur ordinarily during this period was important. During the 2 days prior to opening the tapping cut no variation in resistance more than 1,000 ohms occurred within any half-hour period. These relatively small variations were invariably evident as decreases in resistance. An average variation per pair of terminals amounted to about 250 ohms, although the Bouyoucos Bridge was not accurate to less than 500 ohms at the range employed. In all cases these variations can be considered relatively minute and practically within the margin of error of the instrument.

TABLE I

RESISTANCE OFFERED TO AN ELECTRIC CURRENT BY BARK TISSUES BEFORE AND AFTER TAPPING *Hevea brasiliensis*

DISTANCE OF TERMINALS BELOW TAPPING CUT	PRE-TAPPING RESISTANCE	PERIOD AFTER TAPPING IN MINUTES					
		1–2	5–7	9–12	14–19	21–24	25–28
inches	*ohms*	*ohms*	*ohms*	*ohms*	*ohms*	*ohms*	*ohms*
6	18,500	21,100	22,000	23,000	21,500	20,000	20,000
12	22,000	24,500	25,000	24,000	23,000	21,500	21,000
18	13,000	13,000	13,500	13,000	12,500	12,000	12,000
24	14,000	14,000	14,000	14,000	13,500	13,000	13,000

On the third day the tapping cut was opened to within 1 mm. of the woody tissues. Tapping was done daily thereafter between 7:30 and 8:00 A.M. Measurements made the next 3 or 4 days cannot be considered typical because this period in tapping is required before the tree yields a more-or-less constant amount of latex. Even so, differences in resistances during this period were found comparable to later measurements. Probably the reason early measurements were comparable with those made after tapping for several days was because of the relatively low yields of latex (8 to 12 ml.). Exudation of latex generally ceased within 15 to 20 minutes.

The data for the first four pairs of terminals below the tapping cut on the fifth day of tapping are presented in table I as typical results of the electric measurements. These data are arranged in groups according to the period of time which had passed after tapping. The tapping operation itself only required 15 to 20 seconds. Variations from the pre-tapping resistance offered between each pair of terminals are plotted in figure 2. Variations in resistances on the opposite side of the trunk were found to be so small that no definite effect of tapping was detectable, and a similar lack of variation

was evident in resistances between terminals placed 30, 36, and 42 inches below the tapping cut. Therefore, for the sake of simplicity, data of these terminals are not included.

Discussion

The data in table I and figure 2 show that the resistances of the bark tissues increased after tapping in the immediate area beneath the tapping cut. Since the usual variation in resistances was always a decrease on trees not tapped, the increase of resistance in the immediate area of the tapping cut was concluded to have been caused by tapping. These data also show that the effect of tapping was progressively less at 6-inch intervals below the cut. In fact, no definite effect was measurable 24 inches below the cut, and there was only a slight effect 18 inches below the cut. Therefore, tapping

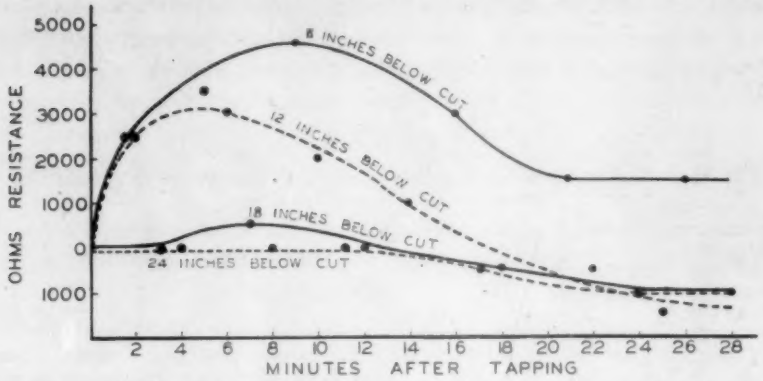

FIG. 2. Variations from the initial resistance to an electric current of bark tissue after tapping *Hevea brasiliensis*. The zero level of the ordinate represents the initial resistance before tapping.

this particular panel was concluded to have affected the bark tissues to a distance of 18 to 24 inches below the cut and to have had no influence on the opposite side of the tree. It is again emphasized that the yields of this seedling tree were relatively low. Thus, these specific results are not applicable to selected clonal trees which often average 75 ml. or more of latex per tapping by the same system. Certainly, the amount of bark influenced by tapping a high-yielding tree would be greater than the amount affected on the seedling tree described here.

A hypothetical explanation of these results is based upon the factors which are associated with the movement of latex. It is probable that the exudation of latex and consequent loss of water caused an increased diffusion pressure in the remaining cell contents to such an extent that resistance was increased. The fact that the effects of tapping were progressively less at 6-inch intervals below the tapping cut is conceivable from a physical standpoint. This method appears to be more exact for defining the area of bark influenced by tapping than previous methods because it measures the vertical declination of tapping effects.

It was further concluded that two cuts in echelon might be safely separated by a distance of 24 inches on the seedling tree studied. The chances of one cut, particularly the high panel, having an area of bark affected by tapping which would overlap that of the lower cut is remote. Thus Brown Bast caused by two cuts being placed too near each other would be avoided. In a few cases Brown Bast is evident from the time of opening the cut. In these instances the inherent characteristics of the tree and not over-tapping are fundamental causes. However, the same distance of separation of the cuts would not apply to high-yielding trees and determinations with them need to be done during periods of their greatest yields. In addition, the lateral areas of bark affected by tapping as well as the distance above the tapping cut are worth further investigation.

Summary

1. The importance of defining the area of bark influenced by tapping *Hevea brasiliensis* is discussed.

2. An electrometric method is described for measuring the area of bark below a tapping cut which is influenced by tapping.

3. The effects of tapping a low-yielding seedling tree with a half-circumference cut were found measurable to a distance between 18 and 24 inches below the tapping cut.

4. Similar measurements need to be done for high-yielding clonal trees during the periods of their greatest production.

FEDERAL EXPERIMENT STATION
U. S. DEPARTMENT OF AGRICULTURE
MAYAGUEZ, PUERTO RICO

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BRIEF PAPERS

ENTRANCE OF WATER INTO SUBERIZED ROOTS OF TREES

RUTH M. ADDOMS

Results of several investigators (**1, 2, 3, 4**) suggest that the absorbing area of root systems may not be restricted to young roots and the young terminal portions of older roots, but that older suberized portions may account for a considerable amount of the water absorbed. By means of potometers HAYWARD, BLAIR, and SKALING (**2**) demonstrated an appreciable amount of absorption by suberized roots of sour orange. KRAMER (**3**) has made a comparative study of rate of absorption by suberized roots of several different species. The present experiment was undertaken to determine the avenues of entrance of water into suberized roots.

Material and methods

Suberized roots were immersed in a dye solution and subjected to vacuum; after this treatment they were studied microscopically. The dye that proved most useful was azosulfamide ("Neoprontosil") in a concentration of 1:1000 in water. Treatments lasted 30 minutes; little or no change was noted as a result of longer treatment. Vacuum was obtained by the use of a filter pump.

Roots used were from young potted trees of yellow poplar (*Liriodendron tulipifera* L.), sweet gum (*Liquidambar styraciflua* L.), and large forest trees of shortleaf pine (*Pinus echinata* Mill.). Roots or root-segments were 3–6 inches long, $\frac{3}{8}$–$\frac{3}{4}$ inches in diameter, and 2–4 years of age. Since preliminary experiments had shown that little or no absorption by suberized roots occurs when young branch roots are present, any young branch roots that were present were not allowed to dip into the solution; in many instances the root-segments used were free from branch roots. Distal cut ends of roots were sealed with grafting wax to minimize the entrance of air and were not allowed to dip into the solution. Thus any colored solution absorbed by the roots must have entered through old roots. The experiment was repeated at least twenty times with yellow poplar and shortleaf pine; at least ten times with sweet gum.

After treatment, roots were dissected under low power binoculars and studied in cross section under a compound microscope. The red color of the dye could be seen easily in the root tissues of yellow poplar and sweet gum. In shortleaf pine, some roots contained so much natural red color in their bark that the color of the azosulfamide could not be detected. In such roots it became necessary to use other dyes. Light green and aniline blue proved reasonably satisfactory.

Results

Microscopic study of yellow poplar roots after treatment showed the presence of appreciable quantities of dye in the bark, while the wood re-

mained uncolored. The bark of yellow poplar is thick with a thin, fairly smooth periderm containing many lenticels. The accumulation of dye was observed in patches, not in uniform concentration throughout the bark. These patches could be traced to three sources: (a) lenticels, (b) breaks around the bases of branch roots, and (c) wounds.

The bark of pine roots is proportionately thinner than that of yellow poplar, with many overlapping plates of brittle periderm several inches long and about $\frac{1}{8}$ inch wide. Some roots are invested with a fungus mantle; in these the plates of periderm seem much less brittle. When they were immersed in a dye solution under vacuum, suberized pine roots absorbed very little dye through the bark. Dissection showed that it tended to enter under the edges of the plates of periderm, but that the overlapping was sufficient to prevent the entrance of much dye solution into the living portion of the bark. When the plates of periderm were removed before treatment, large amounts of dye entered. Lenticels were not observed.

In every pine root studied, the presence of dye in the living portion of the bark could be traced to a small wound previously unnoticed. Little or no entrance was apparent around branch roots. This difference from yellow poplar seems to be the result of more complete coverage of the break in the old root around a branch root by overlapping layers of cork. Around the stumps of dead branch roots or the tips of abortive roots, a complete seal is effected by the formation of periderm. A similar seal has been described by PRESTON (**5**) for lodgepole pine.

A few observations of sweet gum suggest that in amount of absorption through suberized roots it lies between yellow poplar on the one hand and shortleaf pine on the other. Entrance seems to occur through wounds and in some degree around branch roots but not appreciably through lenticels. A more complete study of sweet gum roots is contemplated.

Discussion

The old concept of absorption through only the young portions of root systems has seemed to several investigators (**2, 3, 4**) inadequate to account for the persistence of plants that transpire for long periods during which little or no elongation of roots occurs and when few, if any, young roots are present. Absorption through older suberized roots has been demonstrated experimentally: for sour orange by HAYWARD, BLAIR, and SKALING (**2**); and for dogwood, yellow poplar, and shortleaf pine by KRAMER (**3**). It seems likely that such absorption is important in the field, not only in evergreens but in a wide variety of deciduous trees and shrubs. The results of KRAMER (**3**) indicate that there are great differences in species as to rate of absorption through suberized roots.

An important factor in the potential rate of absorption by a given species is the anatomy of its roots. Since suberin is impermeable to water, a relatively thick suberized layer would render the root impervious to water except where breaks occurred in the suberized layer. Natural breaks occur (a) in

lenticels, (b) around the edges of overlapping plates of periderm, and, in most species, (c) around branch roots. In addition, wounds of various kinds may provide breaks in the suberized layer. The present experiments indicate that yellow poplar, through its lenticels and around its branch roots, absorbs water much faster than shortleaf pine which has few, if any, lenticels and which has an effective seal around its branch roots. These observations confirm the quantitative measurements of absorption by KRAMER (**3**).

Summary

1. Entrance of water into suberized roots of yellow poplar, shortleaf pine, and sweet gum was studied by means of colored solutions. Roots were immersed in the solutions under vacuum, and the appearance of the dye in root tissues was taken as evidence of the entrance of water.

2. Microscopic examination showed that considerable quantities of dye, and therefore presumably of water, could enter old suberized roots and that there was an appreciable difference between different kinds of trees in the amounts that enter.

3. Much more dye was observed in yellow poplar than in shortleaf pine. Preliminary observations on sweet gum indicated that in amount of dye that enters suberized roots, it is intermediate between yellow poplar and shortleaf pine.

4. Three avenues of entrance were observed: (a) lenticels, (b) breaks around branch roots, and (c) wounds.

5. Comparative studies of roots of yellow poplar and shortleaf pine suggest that differences between kinds of trees as to capacity for absorption through suberized roots are attributable in part to anatomical characteristics of the bark of the roots, especially the number of lenticels and the completeness of the coverage by periderm of the inner, living portion of the bark.

DEPARTMENT OF BOTANY
DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

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NOTES

Basil Elijah Gilbert.—Dr. Gilbert died June 27, 1945. He was born at Shedden, Ontario, Canada, on June 25, 1892. He was graduated from McMaster College in 1916 and subsequently served as an officer in the Canadian Expeditionary Forces during World War I. After the war he completed graduate work for the Master's degree at McMaster College in 1920. Dr. Gilbert served as instructor of biology at Brandon College from 1921 to 1924. He then entered the University of Chicago and was awarded the doctorate in plant physiology in 1925. After graduation he joined the staff of the Rhode Island Agricultural Experiment Station at Kingston as a chemist. Since 1928 he served as director of research and was vice director of the station at the time of his death. Dr. Gilbert published numerous important papers on phytochemistry, the nutrition of field crops and the inter-relationships of temperature and photoperiod. He was one of the original members of the American Society of Plant Physiologists and also belonged to the American Association for the Advancement of Science, the American Chemical Society, and the Botanical Society of America.

Frontiers in Chemistry.—Volume IV. Major Instruments and their Applications to Chemistry. Edited by R. E. Burk and Oliver Grummitt. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 151 pages. **$3.50.**

This is the fourth volume of the Frontiers in Chemistry lectures published under the auspices of Western Reserve University. With authoritative discussions by prominent scientists in the industrial field, the volume contains important contributions to chemical literature in the following subjects; electron diffraction and the examination of the surfaces; the electron microscope and its application; x-ray diffraction and its applications; chemical spectroscopy; the application of absorption spectra to chemical problems; and the infrared spectrometer and its application. Each article is expertly handled with figures and charts to elucidate the material. Complete bibliographies are also included with each chapter.

Industrial Oil and Fat Products.—Alton E. Bailey. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 735 pages, 111 figures. $10.00.

The material in this monograph is well organized and concisely yet completely presented. With preliminary chapters on the structure and composition, the reactions, and physical properties of fats and fatty acids, subsequent chapters discuss specific fats and oils as to their characteristics and industrial utilizations. These include materials used in cooking and

salad oils, the plastic-shortening agents, butter and margarine, paints and varnishes, soaps, and other surface-active materials.

The final unit of the book comprises consideration of processes in fat technology. These include the extraction of fats and oils; refining and bleaching; deodorization; hydrogenation; soapmaking; fractionation of fats and fatty acids; fat-splitting, esterification, and interesterification; polymerization; isomerization; solidification; homogenation; and emulsification. Although the book is primarily for chemists, much important information concerning plant oils and their extraction will be of interest to physiologists.

Physical Methods of Organic Chemistry.—Volume I. Edited by ARNOLD WEISSBERGER. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 736 pages. $8.50.

This volume has been prepared to aid the organic chemist in his research by eliminating the often burdensome task of searching periodicals for many physical methods necessary in his work. The book provides a description of tested methods, the theoretical background for understanding them, and the information necessary for a critical evaluation of the experimental results.

The chapters comprise contributions from twenty-seven scientists connected with leading research laboratories and universities. The subjects covered are melting and freezing temperatures, boiling and condensation temperatures, density, solubility, viscometry, surface and interfacial tension, monolayers and duplex films, osmotic pressure, diffusivity, calorimetry, microscopy, crystallo-chemical analysis, X-ray diffraction, electron diffraction, and refractometry. Numerous figures and bibliographical references are included with each topic discussed. A second volume by the same publishers will contain further chapters on this same topic with a subject index to both volumes.

Life Membership.—The Executive Secretary-Treasurer wishes to report the purchase of a Life Membership by CAPTAIN JOHN P. DECKER now of the Army Air Forces, Eglin Field, Florida. CAPTAIN DECKER received the Ph. D. degree from Duke University in June, 1942 and entered the Service a month later. He expects to be released soon and plans to return to Duke and continue work on the physiological aspects of pine—hardwood competition in the Piedmont forests. Since receiving his degree, two papers have appeared in PLANT PHYSIOLOGY under his name.

The purchase of a Life Membership or a Patronship is a definite investment in professional achievement and contributes to the welfare of the Society by ultimately increasing the General Endowment. We are happy to welcome CAPTAIN DECKER to our roster of Life Members and trust that his exemplary action may have a positive effect on others.